DELHI COLLEGE OF ENGINEERING

सत्यमेव जयते

LIBRARY

*Class No.*________________

*Book No.*________________

*Accession No.*________________

Borrower is requested to check the book and get the signatures on the torned pages, if any.

DELHI COLLEGE OF ENGINEERING
Kashmere Gate, Delhi

LIBRARY

DUE DATE

For each day's delay after the due date a fine of 50 P. per Vol. Shall be charged for books of General Section and Re 1.00 for text books Section.

Borrower's No.	Due Date	Borrower's No.	Due Date

THE CHEMISTRY AND TECHNOLOGY OF FOOD AND FOOD PRODUCTS

Prepared by a Group of Specialists
under the Editorship of

MORRIS B. JACOBS, PH.D.

Senior Chemist, Department of Health
City of New York

VOLUME I

1944

'ERSCIENCE PUBLISHERS, INC., NEW YORK, N. Y.

Copyright, 1944, by
INTERSCIENCE PUBLISHERS, INC., New York, N. Y.

INTERSCIENCE PUBLISHERS, INC.
215 Fourth Avenue, New York, N. Y.

Printed in U. S. A.
MACK PRINTING COMPANY, EASTON, PA.

PREFACE

The extensive expansion of almost every field in the theoretical and applied sciences has made it very difficult for a single individual to survey any given field adequately. This is indeed true of food chemistry and food technology.

In presenting these volumes, 41 collaborators have tried to prepare a unified, authoritative work by approaching the problem from the point of view that an expert in any selected subject is best qualified to write about that subject. Thus, to cover the various phases of this work, food technologists, chemists, biochemists, bacteriologists, sanitary engineers, public health officers, food inspectors, and entomologists are represented among the contributors. It is our sincere hope that this book will fulfil a need in the literature of this field.

The two volumes are divided into a total of six parts. The first, on fundamentals, deals with the aspects of food chemistry which are common to all foods. The second part concerns the descriptive aspects of particular food groups and includes some account of the history, statistics, definitions, standards, composition, and chemistry of these food groups. In part three, unit operations and processes applicable to most foods are described. Part four deals with the maintenance of sanitary and quality control of foods and food products. In part five, the principal methods of preserving foods are delineated. Part six is concerned with production methods for the principal foods. Throughout the entire book, the role played by adequate nutrition in modern life is stressed.

There is, in certain respects, an overlapping of the subject matter in section two on descriptive aspects and section six on production methods. As a rule, a food group is treated in two separate but closely integrated chapters, each appearing in one of these sections. Where the material concerning a food group did not lend itself to treatment in separate chapters, the resulting single chapter is placed in part two, if more descriptive, and in part six, if more concerned with production.

In a collaborative effort, it is always difficult to avoid duplication. Such duplication has been permitted only when it is needed to maintain the continuity of a chapter or to simplify an explanation.

The collaborators and the editor wish to express their appreciation to those who have been of assistance in revising and correcting manuscript, reading and correcting proof, and preparing the index. We wish also to extend thanks for the permission to use copyrighted material and illustrations. This courtesy is acknowledged at appropriate points in the text.

Brooklyn, N. Y.
May 1944

MORRIS B. JACOBS

NOTE

The opinions expressed in this book are those of the individual authors. There is no presumption that these opinions are the official views of any branch of the Federal, State, or City Government.

LIST OF CONTRIBUTORS TO VOLUME I

Bailey, Alton E., Senior Chemical Technologist, Southern Regional Research Laboratory, U. S. Department of Agriculture, New Orleans, Louisiana. *Edible Oils and Fats* (pp. 564–590).

Baizer, Manuel M., Department of Chemistry, Brooklyn College, Brooklyn, New York. (With Hugo Zahnd), *Lipids* (pp. 88–109); (with Hugo Zahnd), *Amino Acids and Proteins* (pp. 110–184).

Carr, Ralph H., Professor of Agricultural Chemistry, School of Agriculture, Purdue University, Lafayette, Indiana. *Mineral Matters* (pp. 270–299).

Cathcart, William H., Director, A & P Bakeries Laboratory, The Great Atlantic & Pacific Tea Company, New York, New York. *Baking and Bakery Products* (pp. 686–727).

Degering, Ed. F., Professor of Chemistry, Department of Chemistry, Purdue University, Lafayette, Indiana. *The Carbohydrates* (pp. 49–87), *Carbohydrate and Sugar Foods* (pp. 782–809).

Geddes, W. F., Professor of Agricultural Biochemistry, Department of Agriculture, University of Minnesota, University Farm, St. Paul, Minnesota. *Cereal Grains* (pp. 591–685).

Halvorson, H. Orin, Professor of Bacteriology, University of Minnesota, Minneapolis, Minnesota. *Food Spoilage and Poisoning* (pp. 356–392).

Jablonski, C. F., General Referee on Coloring Matters in Foods for the American Official Agricultural Chemists, U. S. Food and Drug Administration, New York, New York. *Coloring Matters* (pp. 300–326).

Jacobs, Morris B., Senior Chemist, Bureau of Food and Drugs, Department of Health, New York, New York. Editor; *Introduction* (pp. 1–8); *Dairy Products* (pp. 393–434).

Lee, F. A., Assistant Professor of Chemistry, New York State Agricultural Experiment Station, Division of Chemistry, Geneva, New York. *Vegetables, Nuts, Fruits* (pp. 728–781).

Pennington, Mary E., Handling Transportation and Storage of Perishables, Application of Refrigeration, New York, New York. *Poultry and Eggs* (pp. 522–563).

Rosenberg, Hans R., Research Chemist, Jackson Laboratory, E. I. du Pont de Nemours & Company, Inc., Wilmington, Delaware. *Vitamins, Vitagens, Hormones* (pp. 229–269).

St. John, J. L., Head of Division and State Chemist, Division of Chemistry, Agricultural Experiment Station, State College of Washington, Pullman, Washington. *Physical Chemistry* (pp. 9–48).

Schoen, Martin, formerly Research Chemist, Rockwood and Co., Brooklyn, New York; now Teacher of Applied Chemistry, High School of Commerce, New York, New York. *Confectionery and Cacao Products* (pp. 810–839).

Stansby, Maurice E., Technologist in Charge, Fishery Technological Laboratory, U. S. Fish and Wildlife Service, Seattle, Washington. *Fish, Shellfish, Crustacea* (pp. 473–521).

Stern, Kurt G., Chief Research Chemist, The Overly Biochemical Research Foundation, New York, New York. *Enzymes* (pp. 185–228).

Ukers, William H., Editor, *The Tea & Coffee Trade Journal,* New York, New York. *Coffee and Tea* (pp. 840–875).

Urbain, Walter M., Head, Physics and Physical Chemistry Division, Research Laboratories, Swift & Company, Union Stock Yards, Chicago, Illinois. *Meat and Meat Products* (pp. 435–472).

Wilhelmi, Alfred E., Assistant Professor of Physiological Chemistry, Yale University School of Medicine, New Haven, Connecticut. *Digestive Processes* (pp. 327–355).

Worrell, Lee, Instructor in Pharmacy, College of Pharmacy, University of Michigan, Ann Arbor, Michigan. *Flavors, Spices, Condiments* (pp. 876–901).

Zahnd, Hugo, Assistant Professor, Department of Chemistry, Brooklyn College, Brooklyn, New York. (With Manuel M. Baizer), *Lipids* (pp. 88–109); (with Manuel M. Baizer), *Amino Acids and Proteins* (pp. 110–184).

CONTENTS

CHAPTER PAGE

Preface v

List of Contributors vii

PART I—FUNDAMENTALS

I. **Introduction** (JACOBS) 1

II. **The Physical Chemistry of Foods** (ST JOHN) 9

- I Physical Properties 10
  - 1 Density 11
  - 2 Refractive Index 13
  - 3 Optical Rotation 17
  - 4 Viscosity and Plasticity 19
  - 5 Surface and Interfacial Tension 23
- II Hydrogen-Ion Activity 25
- III Colloidal Chemistry of Foods 35
  - 1 Classes of Colloids 35
  - 2 Methods of Preparation 36
  - 3 Properties 36
  - 4 Gels and Sols 39
  - 5 Imbibition 40
  - 6 Emulsions 41
  - 7 Foams 45
  - 8 Other Edible Emulsions 46
- IV Foods as Solutions and Mixtures 47

III. **The Carbohydrates** (DEGERING) 49

- I Definition and Formulation 49
- II Occurrence and Uses 51
- III Structure of Monosaccharides 53
- IV Structure of Disaccharides 62
- V Nomenclature 64
- VI Commercial Sources 73
- VII Laboratory and Special Syntheses 76
- VIII Physical Properties 78

CHAPTER PAGE

III, contd.

IX Reactions of the Reducing Sugars 79
X Reactions of the Di-, Tri-, and Polysaccharides 85

IV. **Lipids** (BAIZER and ZAHND) 88

I Introduction 88
II Classification 88
III Simple Lipids 89
  1 The Fats and Oils 89
  2 Waxes 98
IV Complex Lipids 100
V Some Biological Aspects of Lipid Chemistry 103
  1 The Role of Lipids in Nutrition 103
  2 Lipid Metabolism 105
  3 Biological Synthesis of Lipids 107

V. **Some Aspects of the Chemistry of Amino Acids and Proteins** (ZAHND and BAIZER) 110

I Introduction 110
II Classification of Proteins 111
III Isolation of Pure Proteins 114
IV Hydrolysis of the Proteins 115
V Classification of the Naturally Occurring Amino Acids 119
VI Isolation of the Amino Acids 123
VII Synthetic Methods 128
VIII Some Properties of Natural Amino Acids 139
IX Some Properties of Peptides and Diketopiperazines 146
X The Chemical Nature of Some Normally Occurring Physiologically Active Substances 148
XI The Chemical Nature of Antigens and Antibodies 150
XII Analytical Procedures 151
XIII Molecular Weights of Proteins 157
XIV Structure of Proteins 163
XV Conjugated Proteins 166
XVI Proteins and Amino Acids in Nutrition 177
XVII Recent Developments 182

VI. **Enzymes** (STERN) 185

I Introduction 185
II Enzymes as Catalysts 186
  1 Kinetics of Enzyme Reactions 188
  2 Specificity 210
III Enzymes as Chemical Compounds 213
  1 Enzymes as "Simple" Proteins 214
  2 Enzymes as Conjugated Proteins 216
  3 Structure and Function of Coenzymes 219
  4 Relationships between Enzymes, Vitamins, and Hormones 224
  5 Classification of Enzymes 226

CHAPTER PAGE

VII. Vitamins, Vitagens, and Hormones (ROSENBERG) 229

I. The Vitamins 229
1. The Group of Vitamins A 230
2. Vitamin B_1—Thiamine 234
3. Vitamin B_2—Riboflavin 237
4. Vitamin B_6—Pyridoxine 239
5. Nicotinic Acid and Nicotinamide 240
6. Pantothenic Acid 242
7. Inositol 243
8. *p*-Aminobenzoic Acid 243
9. Vitamin C—Ascorbic Acid 244
10. The Group of Vitamins D 245
11. The Group of Vitamins E 250
12. Vitamin H—Biotin 252
13. The Group of Vitamins K 253
14. Vitamin P 254
15. Nonidentified Vitamins 255
II. The Vitagens 255
1. Essential Fatty Acids 256
2. Essential Amino Acids 256
3. Essential Carbohydrates 258
4. Choline and Related Compounds and the Essential, Transferable Methyl Group 259
5. Essential Organic Sulfur-Containing Compounds 260
III. The Hormones 260
1. The Pituitary Hormones 261
2. The Androgenic Hormones 262
3. The Estrogenic Hormones 263
4. The Corpus Luteum Hormone 265
5. The Hormones of the Adrenal Cortex 266
6. The Hormone of the Adrenal Medulla 267
7. The Hormone of the Thyroid 267
8. The Hormone of the Parathyroids 268
9. The Hormone of the Pancreas—Insulin 268
10. Some Less Well-Known Hormones 269

VIII. Mineral Matters and Other Inorganic Food Adjuncts (CARR) 270

I. Introduction 270
II. Calcium and Phosphorus 276
III. Magnesium 280
IV. Sodium, Potassium, and Chlorine 281
V. Sulfur 283
VI. Iron 284
VII. Iodine 286
VIII. Micronutrients 288
IX. Group Effects of Minerals in the Animal Body 294
X. Soil Factors Determine Nutritional Quality of Food 297
XI. Vitamin and Mineral Fortification of Food 297

CHAPTER PAGE
IX. Coloring Matters in Foods (JABLONSKI) 300

I. Pigments and Lakes 300
II. Coal-Tar Dyes 301
III. Natural Organic Coloring Matters 318

X. The Digestion and Fate of Foodstuffs (WILHELMI) 327

I. Nature and Site of the Digestive Processes 327
II. Salivary Digestion 329
III. Gastric Digestion 330
IV. Intestinal Digestion 337
V. Absorption 346
VI. The Functions of the Large Intestine 353

XI. Food Spoilage and Food Poisoning (HALVORSON) 356

I. Introduction 356
II. Biochemical Factors Causing Spoilage 357
III. Conditions Affecting Spoilage 363
IV. Enzyme Spoilage 373
V. Oxidative Changes 374
VI. Prevention of Spoilage 375
VII. Analysis of Foods for Spoilage 379
VIII. Food Poisoning 380

PART II—FOODS

XII. Milk, Cream, and Dairy Products (JACOBS) 395

I. Milk 395
II. Cream 418
III. Butter 422
IV. Evaporated Milk 424
V. Concentrated Milk 425
VI. Condensed Milk 425
VII. Ice Cream 426
VIII. Milk Sherbets and Ices 427
IX. Cheese 428
X. Skim Milk 430
XI. Buttermilk 430
XII. Fermented Milk 431
XIII. Whey 432
XIV. Dried Milk Products 432
XV. Malted Milk Powder 433
XVI. Filled Milk 433
XVII. Imitation Milk 434
XVIII. Milk Beverage 434

XIII. Meat and Meat Products (URBAIN) 435

I. The Raising and Marketing of Livestock 435
II. The Operations of the Meat-Packing Industry 438

CHAPTER PAGE
III. Statistics on Meat Production and Consumption........ 439
IV. Bureau of Animal Industry Definitions........ 441
V. Classes and Grades of Meat........ 441
VI. Composition of Meat........ 445
VII. Post-Mortem Changes in Meat........ 447
VIII. Preservation of Meat........ 450
IX. Freezing of Meat........ 451
X. Cured Meats........ 452
XI. Sausage........ 460
XII. Canned Meats........ 461
XIII. Dehydrated Meats........ 461
XIV. Meat Cookery........ 462
XV. Nutritional Aspects of Meat........ 464
XVI. Food Poisoning Resulting from Meat........ 464
XVII. Food and Feed By-Products of Meat........ 465
XVIII. Inedible By-Products........ 470

XIV. Fish, Shellfish, and Crustacea (STANSBY)........ 473

I. Description and Extent of Commercial Fisheries........ 473
II. Composition of Fish........ 481
III. Spoilage of Fish........ 493
IV. Fishing Methods........ 494
V. Handling Fresh Fish........ 497
VI. Handling Frozen Fish........ 501
VII. Canned Fish........ 504
VIII. Smoked Fish........ 510
IX. Salted and Dried Fish........ 511
X. Fish By-Products........ 514
XI. Shellfish and Crustacea........ 516
XII. Bibliography of Fishery Technology........ 519

XV. Poultry and Eggs (PENNINGTON)........ 522

I. Poultry........ 522
II. Dressing Poultry in the Packing Plant........ 528
III. Eggs........ 540

XVI. Edible Oils and Fats (BAILEY)........ 564

I. The Nature of Oils and Fats........ 564
II. Sources and Production of Oils........ 570
III. Examination and Evaluation of Oils........ 572
IV. Characteristics........ 581
V. Utilization........ 585

XVII. Cereal Grains (GEDDES)........ 591

I. History, Production, Structure, Classification, and Storage Properties........ 591
II. Chemical Composition of the Cereal Grains........ 615
III. Barley........ 627
IV. Buckwheat........ 628
V. Corn or Maize........ 632

CHAPTER PAGE

XVII, contd.

VI Oats 637
VII Rice 639
VIII Rye 646
IX Wheat 649
X Cereal Preparations 677
XI Cottonseed, Peanut, and Soybean Flours 680

XVIII. Baking and Bakery Products (CATHCART) 686

I Progress of the Baking Industry 686
II Processes for Yeast Raised Bakery Products 688
III Gas Production in Yeast-Raised Doughs 689
IV Gas Retention in Yeast-Raised Doughs 694
V Sour Rye Bread 698
VI Cake and Cookie Production 700
VII Development of Staleness 706
VIII Nutritive Properties of Bakers' Products 709
IX Microbiology of Bakery Products 712
X "Bleeding" Bread 719
XI Mold on Bakery Products 719
XII Scoring of Bakery Products 723
XIII Packaging 726

XIX. Vegetables, Mushrooms, Nuts, and Fruits (LEE) 728

I Vegetables 728
  1 Bulbs 728
  2 Cucurbits 729
  3 Flowers 731
  4 Fruits (except Cucurbits and Legumes) 732
  5 Leaves and Stems 737
  6 Legumes 742
  7 Roots 748
  8 Shoots 751
  9 Tubers 752
II Mushrooms and Other Fungi 756
III Nuts 759
  A Domestic Nuts 759
  B Tropical and Subtropical Nuts 761
IV. Fruits 762
  1 Berries (except Grapes) 763
  2 Citrus Fruits 766
  3 Drupes 769
  4 Grapes 772
  5 Melons 773
  6 Pomes .. 774

XX. Carbohydrate and Sugar Foods (DEGERING) 782

I Historical Development 782
II Carbohydrates Used as Foods 786
III The Digestion of the Carbohydrates and Sugars 795

CHAPTER PAGE
IV The Anabolism of the Simple Sugars 796
V The Catabolism of Glycogen and D^+-Glucose 797
VI The Interrelationship of Carbohydrates, Fats, and Proteins 806

XXI. Confectionery and Cacao Products (SCHOEN) 810

I Historical Background 810
II Ingredients in Candy 814
  1 Food Value 814
  2 Sugars and Sirups 814
  3 Pectin and Other Gums 819
  4 Gelatin 823
  5 Cacao 824
  6 Confectionery Flavors 835
III Candy as a Food 837

XXII. Coffee and Tea (UKFRS) 840

I Coffee 840
II Tea 856

XXIII. Flavors, Spices, and Condiments (WORRELL) 876

I Spices 870
II Flavoring Extracts 896
III Vinegar 893
IV Mayonnaise and Salad Dressings 908

Subject Index 903

PART I

FUNDAMENTALS

Chapter I

INTRODUCTION

MORRIS B. JACOBS

Brooklyn, New York

The three necessities for the preservation and maintenance of life are food, clothing, and shelter. Of these three, as civilization progressed, man provided for his needs of clothing and shelter with a greater degree of success than for his need of food. Indeed, one might say that with the development of civilization our food habits deteriorated and that some aborigines were more adequately fed than civilized peoples. This, as a general rule, is no longer true. It has been only in comparatively recent times, in a historical sense, that the full aspects of the problem of providing proper food for man have been realized. Nor have the problems of proper clothing and housing been completely solved, but for many years they were far advanced with respect to the food problem

In ancient times, long before the industrial revolution and the marked shift from rural to urban life, when men were hunters, fishermen, and farmers, they obtained their food directly from nature and were more certain of having a balanced diet than they are today when even farming is often a one-crop business. As Monier-Williams[1] points out, in comparing the food supply of the present day with that of olden times, there are several important factors to be taken into consideration. The range of foodstuffs available for primitive man was small for it was restricted to cereals, fruit, herbs, oil, honey, milk, meat, and fish, all of which were produced almost at his door. Sometimes he had a great abundance. At other times, there was great scarcity but always his foods were prepared for use by the simplest methods. With the advance of civilization, men tended to congregate in cities and to abandon pastoral for industrial occupations; consequently it became necessary to arrange for a daily supply of food to be brought in from the surrounding country. This did not present any great difficulty for communities of moderate size, situated in the midst of fertile agricultural districts, but with the gradual growth of great industrial centers, the provision of an adequate and sufficiently varied supply of sound and wholesome food proved to be no easy task.

The interest of man in food arises primarily from the hunger instinct for the preservation of life. But the hunger instinct can no longer be relied upon as an adequate guide for man. Even in primitive and biblical times, the hunger instinct had to be conditioned and was conditioned by rules. One of these primitive taboos was the rule against eating meat torn in the field, probably based on the sanitary principle that the carrion might be decomposed and poisonous.

---

[1] G W Monier-Williams, *Chemistry in Relation to Food* Benn, London, 1924.

With the Hebrews this taboo was elaborated into the entire code of ritual slaughter, one of the earliest systems of sanitary significance. In a similar manner the taboo against cooking a young goat in its mother's milk was developed into the Kosher rules observed by Jewish housewives. The prohibition against eating shellfish probably arose from the observation by Moses that they throve in unclean waters. The dietary regulations of other religions, such as the Hindu rule against eating beef—for to them the cow is sacred (all animals being more or less sacred)—spring from similar sources.

We know we must have food to eat in order to live. In more recent times we have learned that we need not only sufficient food for living but also proper food for adequate living. Undernourishment is one of the greatest causes of illness. It is shocking to think that some are undernourished, not because of lack of money, but because of ignorance and, at times, even stupidity.

1. Functions of Food

Food has three chief functions: First, it serves as fuel for the body, providing heat by a process of slow oxidation; second, it furnishes the building material for growth and repair; and third, it provides for the regulation of the body functions. In infancy, childhood, and youth, the growth function is of greater importance than in later years, but all through life the entire body is renewed and repaired.

2. Essential Food Factors

We have been taught that an adequate food supply depends upon several factors. These may be grouped as follows:

1. Enough protein material to supply an adequate amount of the necessary amino acids.
2. Sufficient digestible organic material (carbohydrate, fat, and protein) to yield the required energy.
3. Adequate quantities of the vitamins needed by human beings.
4. Adequate quantities of the vitagens needed by human beings.
5. Mineral matters in sufficient quantity and correct proportion.
6. Adequate amounts of good water and air.

These factors are discussed in subsequent portions of the book.

3. Science, Chemistry, and Technology

A science is a branch of study concerned with observed facts systematically classified and more or less colligated by being brought under general laws. Chemistry is the science which deals with the composition and properties of substances and the changes they undergo. In a like manner, food chemistry is the science which deals with the composition and properties of foodstuffs and the changes they too undergo. Technology is the systematic classification of the knowledge of the industrial arts, especially of the more important manufacturing fields. Thus the technology of food is concerned with the scientific study of food and food preparation as industrial processes.

The application of the scientific principles of chemistry, bacteriology, and engineering to industrial practice has resulted in a marked advance in the science of food technology. In its fundamental aspects, the processing of foods does not differ from the processing of other organic materials derived from natural sources. In its basic concepts, food technology deals with the processing and production of foods of uniform quality on a large scale. The best use of new types of plant equipment, of processing, and of distribution which have been developed in the past few decades requires large economic units.[2] To ignore this point is to miss an important phase in the development of food processing.

Within the past five or six decades great advances have been made in the application of scientific knowledge to the preparation, processing, preservation, and transportation of foods so that it is possible to have foods in season all year round. The methods of production of our staple foods, namely, cereals, milk, meat, fish, fruit, and vegetables are much the same as in former years despite vast improvement in the methods with consequent increase in yield but it is in their distribution, packaging, and the variety of processed foods produced and developed that great strides have been made in our times.

4. History

We are prone to think that it was at the turn of the century that scientists used to distinguish rather closely between pure science and applied science. These pure scientists pursued knowledge only for esoteric reasons and were horrified at the thought that practical use might be made of their discoveries. But other scientists, practical men, tried hard to develop applications which would be the answer to human needs and thus the science of food technology is the result of their labor.

Actually the food industry has its roots in the remote past. Almost every major subdivision of the food industry and particularly those dealing with grain and bread, meat and meat products, fish and fish products, spices and preserved foods, was a well-defined trade guild. These guilds had special prerogatives. Thus the *Garblers* controlled spices. These organizations were closely knit groups which passed on the results of their experience to others within the group. From this experience of generations, food technology developed just as other sciences developed.

If we are to accept as a basis that technological development implies production on a relatively large scale, milling (Fig. 1) and baking (Fig. 2) were well advanced in Roman times. Thus before 171 B.C. bakers did not belong to a trade guild. Afterward, however, public bakeries were established. In Trajan's reign (98–117 A.D.)

---

[2] R. K. Froker, A. W. Colebank, and A. C. Hoffman, U. S. Dept. Agr., *Circ.* **527** (1939).

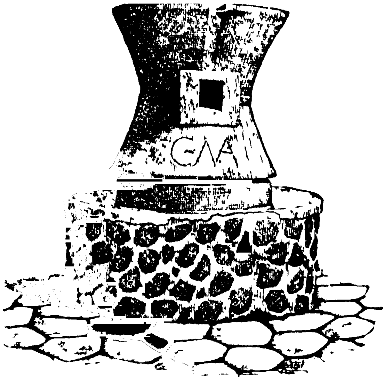


Fig 1.—A Pompeian mill without its framework.[3]

bakers were organized into guilds and bread was distributed to the public daily instead of distributing grain once a month.[3]

Fig. 2 —Bakery with mills.[3]

Other industries developed by the Romans were the production of olive oil and the preservation of olives. The olive fruit was picked, permitted to ferment slightly, and the oil was then pressed out by means of a machine (Fig. 3). This first pressing was permitted to stand in jars to allow the sediment to settle and the supernatant fluid was skimmed off. This was repeated as much as thirty times until the oil was clear. Second and even third pressings were made of the pulp. For small-scale production, this process is still used in Italy.

---

[3] H. W. Johnston, *Private Life of the Romans.* Scott, Foresman, Chicago, revised ed , 1932

Olives were preserved by salting and drying, by immersion in strong brine, in boiled must, or mashed with spices and vinegar.

From the point of view of preservation of foods, some practices are immemorial. Thus freezing or icing of flesh foods was used in all parts of the world where ice was obtainable and where cold weather prevailed at some time during the year. Curing meat by the addition of salt is another method whose origin is shrouded in antiquity. Herodotus (475 B.C.) is quoted as saying, of the Egyptians, "They live on fish, raw, but sun-dried or steeped in brine; they eat also raw quail and ducks and the smaller birds, salted beforehand." The Romans also preserved fish with salt.

Jensen[4] refers to Apicius[5] as one who gave directions for the preparation and preservation of meat and vegetable products.

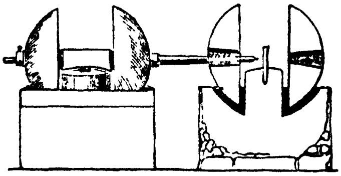


Fig 3 —Olive mill [3]

The Chinese[6] were acquainted, as early as 3000 B.C., if not earlier, with the production of a type of beer from rice after malting and fermentation. In 1900 B.C., a Chinese author described a process for making a wine from fermented rice and also described dough raising in bread making.

Cheese was manufactured by Greeks, Romans, and Jews thousands of years ago, and in all probability by people of more ancient nations. Hippocrates, Aristotle[7] Caesar, and Pliny mention it. Its manufacture probably resulted from the accidental curdling of milk. Aristotle and Hippocrates, however, mention the use of juice of figs and the storage in stomachs. Butter also has an ancient heritage. It was used in the religious rites of Hindus, and the Greeks and Romans used it as an unguent.

It is clear, however, that in all of these instances or in most of them, little of the product was prepared or preserved on a large basis or on a large commercial scale.

(a) *Food Chemistry and Nutrition*

It was little more than 100 years ago that the sciences of food chemistry and nutrition were in their earliest stage of development. These sciences developed along with organic chemistry. Antoine Lavoisier, the eminent French chemist, was one of the first to recognize that food was the fuel of the body. François Magendie, a French physiologist, recognized that foods belonged to groups having different nutritional significance. Although a few analyses of foods were made prior to Liebig's work,[8] with the development by Liebig and his coworkers of organic chemical analysis, the science of food chemistry and analysis was placed on a firm basis.

---

[4] L B Jensen, *Microbiology of Meats.* Garrard, Champaign, 1942

[5] J D Vehling, *Apicius* Hill, Chicago, 1936.

[6] R. Furness, *The Fermentation Industry* Benn, London, 1924.

[7] W. Tibbles, *Foods, Their Origin, Composition and Manufacture* Baillière, Tindall, and Cox, London, 1912

Along with the development of food chemistry from the analytical point of view, came the development of food chemistry from the nutritional point of view with Magendie, Lavoisier, Liebig, Rubner, Voit, Atwater, and Lusk stressing the importance of proteins, fats, and carbohydrates as the essential food materials. It was only in relatively recent times that the role that vitamins, vitagens, and minerals play was fully appreciated. But even in the case of vitamins, there were early observers. One need only refer to the English physician Lind and the Austrian physician Kramer, who in 1757 and 1720, respectively, advocated the use of lemon juice in the treatment of scurvy. This was observed even earlier by Dietz in 1665.[9]

(*b*) *Food Technology*

It is well, however, to point out that food technology itself, as well as production of food as an industry, actually has a history extending as far back as the history of modern chemistry. Of course, it was not called food technology because it was considered a part of chemical technology. Thus Wagner's *Chemical Technology*,[10] which first appeared in 1850, has good coverage in the 8th edition, 1870, of the following: starch, sugar manufacture, cane sugar, beet sugar, grape sugar, fermentation, wine making, beer brewing, spirits, bread baking, manufacture of vinegar and essential oils.

Some examples may make this point clear. In 1747, Marggraf discovered crystals of sugar in the red beet and suggested that it might be possible to extract it on a commercial basis. It was twenty-six to twenty-eight years later that oxygen was discovered (Scheele, 1773; Priestley, 1774; Lavoisier, 1775), and it was not until 1783 that Lavoisier with the aid of LaPlace was finally able to disprove the phlogiston theory by synthesizing water from hydrogen and oxygen.

The preparation of beet sugar from sugar beet was not developed until about 1800 Napoleon I, in furtherance of his "Continental System" which forbade the importation of certain items, among them sugar from sugar cane, that might have been of aid to the British financially, supported a trial of the German method of Achard and Hermbstadt for the manufacture of beet sugar. A commercially feasible method, however, was not developed until 1825, nearly ten years after Napoleon's defeat Wagner[10] states that the annual production of sugar in 1811 did not exceed 13,000,000 lbs. and in 1870 the yearly consumption of beet sugar exceeded 15,000,000,000 lbs This large quantity was supplied by more than eighty manufacturers.

Priestley in 1767 also discovered that "fixed air," as carbon dioxide was termed i

[8] F. Filby, *A History of Food Adulteration* Allen & Unwin, London, 1934

[9] H R Rosenberg, *Chemistry and Physiology of the Vitamins* Interscience, New York, 1942

[10] R. Wagner, *Handbook of Chemical Technology*. Translated by W. Crookes Appleton, New York, 1872.

his time, gave a pleasant, acidulated taste to water. In 1772 he published a pamphlet on this subject entitled, "Impregnating Water with Fixed Air." This was the origin of the manufacture of soda water. Dr. Philip Physick and Townsend Speakman, a druggist, of Philadelphia, founded the carbonated beverage industry in America about 1785.

Thornton[11] points out that the records of the Chinese show that early in the 17th century, cottonseed was sometimes used to produce an oil for illumination. About 1768, Dr. Otto of Bethlehem, Pa., extracted oil from cottonseed and presented samples to the American Philosophical Society. A British society offered a gold medal in 1783 to anyone who could produce one ton of oil and 500 lbs. of cake and a silver medal for half a ton of oil and 200 lbs. of cake but no one ever claimed the medals. Mills were built as early as 1800 but it was not until about 1826 that a mill was built in Columbia, S. C., which yielded good oil. Another was built in Natchez in 1829. Most mills were unsuccessful because of the difficulty of cleaning the seed. The first satisfactory huller was not built until 1852.

Food technology developed in full with the growth of the processing industries and with improvement in food machines, transportation, refrigeration, storage, and packaging. The canning of foods was made practical by Nicolas Appert about 1809. The tin can was first introduced about 1810 but it was not until about 1860–1875, when calcium chloride baths and the steam pressure retort were used, that canning really developed as a full-fledged industry. In the period 1870–1880 the first great strides in refrigeration and storage were made. The development by Gustavus F. Swift of the refrigerator car, first made practical in 1875 by G. H. Hammond, changed the entire perishable food industry. It was only in 1879 that the first comparatively modern roller mill was invented for milling wheat. It has been only in the past few decades that the subject of proper packaging received any attention worth mention.

The notion that the processing of foods was an applied science akin to other industrial and technological studies was difficult to foster but the work of many scientists and food processors has put the science of food technology on a firm foundation.

5. Purpose of Processing Foods

The major purposes for the processing of foods may be summarized as follows:

1. To preserve foods against deterioration.
2. To provide foods of uniform quality on a large scale.
3. To arrange a uniform and adequate supply of foods throughout the year.
4. To create new products of increased appetite appeal or of specific nutritive value.[12]
5. To prepare food combinations for the convenience of those who do not have the time, skill, or opportunity to make them from raw ingredients.[12]

---

[11] M. K. Thornton, "Cottonseed Products." *Oil Mill Gazetteer*, 1932.

[12] Paul E. Howe, *Ind. Eng. Chem.*, **35**, 24 (1943).

6. Aims to Be Achieved

It has been stressed[12] that the use of processed foods may contribute to or be a potential detriment to the health of our nation depending upon their use in the daily diet with a proper regard for their nutritive value.

As has been pointed out by R. C. Newton,[13] the interest of man in food was concerned, first, with obtaining an adequate supply at all times; second, in achieving palatable foods; third, in paying proper attention to their sanitation and freedom from disease; and, fourth, with their nutritive value. Food technology has made great strides in the first three factors. It must make equal progress in the fourth.

We cannot conclude that the technological and scientific advances in the production, processing, and use of foods will solve all problems of nutrition or food preservation for we have the example of the failure of the fat-protein-carbohydrate theory and the failure of the expansion of use of dehydrated foods after the end of World War I because of lack of palatability, but this we do know, such advances should enable us to feed more people better than ever before.

---

[13] R. C. Newton, *Proc. Inst. Food Tech.*, **1942**, p. 7.

Selected Bibliography

Blyth, A. W., and Blyth, M. W., *Foods: Their Composition and Analysis*. Van Nostrand, New York, 1903.

Fischer, F., *Wagner's Manual of Chemical Technology*. Translated by W. Crookes. Appleton, New York, 1912.

Johnston, H. W., *Private Life of the Romans*. Scott, Foresman, Chicago, revised ed. 1932.

Loesecke, H. W. von, *Outline of Food Technology*. Reinhold, New York, 1942.

Monier-Williams, G. W., *Chemistry in Relation to Food*. Benn, London, 1924.

Partington, J. R., *Origins and Development of Applied Chemistry*. Longmans, Green, New York, 1935.

Prescott, S. C., and Proctor, B. E., *Food Technology*. McGraw Hill, New York, 1937.

Sherman, H. C., *Chemistry of Food and Nutrition*. Macmillan, New York, 1941.

Shrader, J. H., *Food Control*. Wiley, New York, 1939.

Stillman, J. M., *The Story of Early Chemistry*. Appleton, New York, 1924.

Tibbles, W., *Foods, Their Origin, Composition and Manufacture*. Baillière, Tindall, and Cox, London, 1912.

Vulté, H. T., and Vanderbilt, Sadie B., *Food Industries*. Chemical Pub. Co., Easton, 1928.

Wagner, R., *Handbook of Chemical Technology*. Translated by W. Crookes. Appleton, New York, 1872.

Chapter II

THE PHYSICAL CHEMISTRY OF FOODS

J. L. St. John
Pullman, Washington

> *The biological organism can be looked upon as a complex system of chemical constituents, composed mainly of proteins, carbohydrates, fats and lipids, mineral elements, and water, which are organized by the mysterious forces which we call "life," and the actions and reactions of this protoplasmic mass are in turn determined by the energy interchanges of molecular transformations and surface and interfacial forces . in the last analysis many of the reactions of biological systems are dependent upon the colloidal phenomena operating in such systems.*
>
> R. A. Gortner (1938)

Animals eat either animal or plant tissues which are composed largely of complex organic substances which are in colloidal condition, and of inorganic materials. The colloidal nature of the principal components of our foodstuffs is essential to the maintenance of a reserve food supply and is of fundamental importance in the storage and distribution of foods. It is also important to maintain intact the colloidal condition of foods because of other reasons, one of which relates to palatability. A mixture of amino acids, which are the primary constituents of protein, is unpalatable and is, in fact, nauseating to the taste. Glutamic acid in pure form even acts as an emetic. While sugars are assimilated in the form of glucose, animals cannot consume enough of such a sweet food to satisfy body needs. Therefore, a large part of the carbohydrate material is supplied to animals in the form of pure starch which is tasteless. Butter is a most palatable food, but its principal constituents, such as butyric, caproic, caprylic, and capric acids, are distasteful and repulsive to the sense of smell. These unpalatable compounds including amino acids, glucose in too large quantities, and fatty acids are representative of the digestive products of the three food components, proteins, carbohydrates, and fats It would thus be impossible to nourish the body with predigested food, because of its unpalatability. Foods in the colloidal condition also exert little or no osmotic pressure in the digestive tract and are not immediately available for absorption. This prevents their rapid entrance into the body fluids in concentration sufficiently great to be harmful.

The colloidal properties of foodstuffs of animal origin such as meats are preserved by refrigeration, by canning in hermetically sealed containers, by salting, and, as more extensively used recently, by drying. Meats are no longer a completely living tissue, but are a surviving tissue in the process of becoming inanimate as a result of progressive enzyme and other deteriorating action. Preservation methods interfere with this degradation process.

Vegetable foods, fruits, and grains are living tissue and naturally retain their vitality for a considerable period. This period may be extended by proper storage conditions. This is particularly necessary in the case of fruits, and to a lesser extent in the case of vegetables. If, however, the physical or colloidal structure of foods such as the potato is damaged by freezing or otherwise, rapid deterioration occurs if the food is not used soon after thawing probably as a result of bringing the enzymes present into contact with the substrate. The frozen potatoes soon become sweet, amino acids are produced from the proteins, and the food thus becomes unfit for consumption in a few hours.

Since agriculture and food manufacture are essentially forms of applied biochemistry, physical chemistry which forms an essential part of this field has made possible great advances of immediate practical usefulness. An increase in our knowledge of the behavior of colloidal materials including emulsions is destined to influence and is influencing profoundly the methods of food production. Wheat owes its pre-eminence as a bread grain in large measure to the fact that it contains a mixture of proteins known as gluten which, with water, forms a tenacious elastic mass. It thus yields dough which, because of its colloidal properties, may be used to produce bread. If we can eventually control the colloidal state to a sufficient extent, it may be possible to give to other proteins beside gluten, the physicochemical properties of gluten. Other grains besides wheat and rye may then be used for bread production.

Butter is an important emulsion of the water-in-oil type. Milk is an emulsion of the oil-in-water type which also contains the other classes of food materials including proteins, carbohydrates, and mineral matter, in addition to certain vitamins, either in the colloidal condition or in solution. Eggs are an excellent example of a natural biocolloid in living condition. Meat is largely protein in colloidal condition. Most of the food industries that elaborate raw materials derived from living matter have to deal with the colloidal condition. Therefore, we can make progress in these arts only as rapidly as colloidal chemistry is applied.

I. PHYSICAL PROPERTIES

Much of the earlier work on food chemistry was concerned with the organic chemistry of food, and energy values. A very large amount of valuable analytical work has also been done on foods to determine the nature and percentage of various constituents. This has served as an important basis for the evaluation of food and as a basis for the regulatory work carried out by federal and state regulatory agencies.

Recent emphasis of physical chemistry in food industries has contributed greatly to advances in this field as demonstrated by improvements in food products, the developments of new products such as frozen foods and more palatable dehydrated foods.

In a study of the physical and colloidal chemistry of foods, a consideration of certain physical concepts and methods such as density, refractive index, optical rotation, viscosity and plasticity, surface and interfacial tension, and hydrogen-ion activity is essential to an understanding of the chemistry and technology of foods. They are an integral part of the physical and colloidal chemistry of foods.

1. Density

The quantity of matter in a unit volume of substance is called the absolute density. The relative density is known as the specific gravity. Water is the standard substance usually used as a basis for comparison, and specific gravity is commonly defined as the relation between the weight of a unit volume of a substance and an equal volume of water at a temperature of 15.5 or 20° C.

(a) *Methods of Measurement*

Pycnometer.—The most accurate method for the determination of specific gravity is to weigh equal volumes of water and the unknown solid, liquid, or solution. A number of types of pycnometers are used for this purpose. They are calibrated by determining the weight of freshly distilled water, which the pycnometer will contain at the temperature to be used for comparison. After drying, the pycnometer is refilled with the substance in question and reweighed at the temperature used, which should be accurately controlled. The volume of liquid within the pycnometer is accurately controlled frequently by using a stopper with a capillary outlet, and often the bottle is stoppered with a thermometer for accurate temperature measurement.

Westphal Balance.—Specific gravity may also be determined by use of an ordinary laboratory analytical balance by suspending a heavy glass sinker or a bulb containing mercury above one balance pan. The bulb is weighed in air and reweighed while submerged in the unknown liquid which is in a container supported on a stand above the balance pan. The specific gravity is calculated from the ratio of the two weights. An adaptation of this method is incorporated into the Westphal balance. The scale on the beam of this balance is calibrated to read directly in terms of specific gravity of the unknown in comparison with water. The bulb used with this balance contains a thermometer.

Hydrometer.—A third method of determining specific gravity is by the use of a hydrometer or a specific gravity spindle. It is quicker and easier in manipulation and sufficiently accurate for the control of many commercial operations. A hydrometer consists of a weighted bulb and a stem which is calibrated in terms of specific gravity, or other scales. Hydrometers are constructed to cover various ranges of specific gravity of solutions or liquids. The reading on the stem at the surface of the liquid indicates the specific gravity. The hydrometer should float freely and must not touch the bottom or walls of the cylinder containing the liquid. Since specific gravity varies with temperature, some hydrometers are constructed with a built-in thermometer so that a correction for temperature variation may be applied.

A number of scales are used with hydrometers, more especially those constructed for use in sugar solutions. A hydrometer designed to read percentages of sucrose directly was first constructed by Balling. The scale in general use at present is the one recalculated by Brix and is usually designated as "degrees Brix." The difference between the Brix and the Balling scales is slight. Another scale used is attributed to Baumé. This instrument is standardized by use of a solution of common salt. The scale reads 0 in distilled water and 15° in a 15% salt solution. The readings on each of these different scales may be converted into terms of any other scale, including specific gravity. Tables are found in several texts and handbooks for this conversion. Hydrometers for specialized uses are available such as alcoholometers for measuring alcohol content, and salometers for salt content.

(*b*) *Use of Density*

The specific gravity of equal concentrations of the different sugars at the same temperature is approximately the same. The specific gravity of a sugar solution varies with the temperature and with the concentration of the solution. With increase in temperature, solutions expand in volume and the specific gravity becomes progressively less. Tables are available in texts and handbooks showing the relation of specific gravity to the concentration of different sugars at a constant temperature. Temperature correction tables are generally included. Such tables for invert sugar solutions are not, however, available at present. The density of freshly prepared solutions of glucose, fructose, and other sugars changes on standing. This is due to the same cause as mutarotation. In determining the density of solutions of such sugars, it is necessary to allow the solution to stand until the mutarotation is complete and the density has reached a constant value.

Specific gravity tables developed for sucrose may be fairly accurate for use with solutions of other sugars and carbohydrates when the solutions are not too concentrated. The use of these tables, however, for impure sugar solutions may lead to serious errors, since the impurities affect the specific gravity and since the percentage of dissolved impurities for the same specific gravity may differ materially from the percentage of sucrose. This is true, for instance, if we compare the percentage of sucrose, tartaric acid, potassium sodium tartrate, and potassium carbonate. For the same specific gravity, these decrease in the order named and the percentage of potassium carbonate is less than one-half the percentage of sucrose while the other percentage values are intermediate.

Solutions too viscous to be used with a hydrometer may sometimes be diluted before taking the specific gravity. The Brix reading of a solution of pure sugars is in direct proportion to the dilution. This, however, is not true with molasses when a higher result is obtained on dilutions because the contraction on dilution is greater with a pure sucrose solution.

Methods have been proposed for determining dry substance in solution by use of the Brix hydrometer. Such methods must be used with great caution because the relationship varies depending upon a number of factors. Such a procedure may, however, be used to detect abnormalities in the composition of sugar products.

2. Refractive Index

(a) *Definition*

Solid substances, liquids, and solutions of many substances exhibit the property of refraction which is a characteristic property. Refractive index is used to determine the identity and purity of various food products and also to determine the percentage of components in foods. It ranks in importance with the specific gravity for these purposes. The index of refraction is primarily of importance in work on oils and sugars.

If a beam of light passes from one medium to another, such as from air into water, it does not continue in a straight line but its direction is changed or it is bent or deflected from its original course. This is true if the second medium has a different optical density from the first. Optical density must not be confused with the idea of physical density of a material, since the two do not correspond. A good illustration of the result of refraction may be observed when a knife or an oar is seen partly immersed under water. The bent appearance is due to refraction of light.

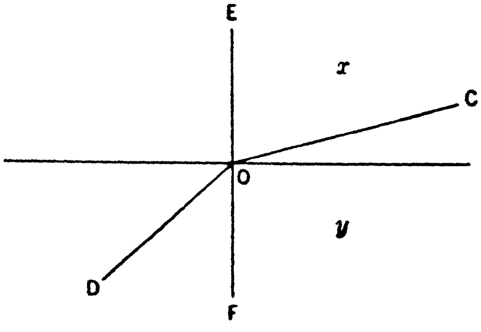


Fig. 4.—Law of refraction.

In Fig. 4, let x and y represent two media, air and water. CO represents a beam of light striking the surface of the water at O at an angle. In passing into the water, the beam is refracted, that is, it is bent as it passes into the water. EF is a line perpendicular to the surface of the water, constructed through the point O. Since water is optically more dense than air, a beam of light, OD, is deflected toward the perpendicular or the normal EF The angle DOF between is the angle of refraction and is smaller than the angle COE, which is the angle of incidence in air. The ratio of these two angles, that is, COE and DOF, is the basis for the calculation of the index of refraction. The ratio of the sine of the angle of incidence to the sine of the angle of refraction is called the index of refraction. The ratio of sine I over sine R equals N. This is also the ratio of velocity of light in the two media.

(b) *Variation with Wave Length*

The refractive index varies with the wave length of the light. It increases from the red toward the violet end of the spectrum. From this it is evident that when ordinary daylight is refracted, it is dispersed into light containing the various colors of the spectrum. Thus, it is necessary to use monochromatic light in measuring the index of refraction, unless a compensator is built into the instrument to overcome the dispersion of light.

(*c*) *Variation with Temperature*

The index of refraction of solutions of all substances varies with the temperature, decreasing as the temperature rises. It is therefore necessary to keep the temperature constant during the measurement of refractive index. Equipment for this purpose is furnished by manufacturers or may be constructed in the laboratory.

(*d*) *Refractometer*

Before using a refractometer, it should be checked with liquids of known refractive index. Freshly distilled water free from air has a refractive index at 20° C. of 1.33299. It is convenient for checking the lower divisions of a refractive index scale. Other liquids for this purpose are furnished with the refractometer.

Abbé Refractometer.—A number of types of refractometer have been developed for the measurement of refractive index of different substances. The Abbé refractometer is generally applicable and is used in determining the refractive index of fats and fatty oils, and in estimating the concentration of sugar solutions. It employs only a few drops of material and the scale is designed so that the refractive index is read directly while using ordinary white light. Monochromatic light may be used. For details regarding the principles and operation of the refractometer, reference is made to standard texts on physics or to detailed descriptions such as those appearing in Browne and Zerban,[1] or in Leach,[2] which discuss in detail the application of different types of refractometers to food work.

An Abbé refractometer consists of two prisms separated by a small space in which a few drops of the liquid or solution to be studied is placed. Light passing through the prisms and the sample is condensed by the optical system and directed to the eyepiece of the instrument. The prisms may be rotated until the border line between refracted and reflected light intersects the cross hairs in the eyepiece. The angle through which the prisms have been rotated is accurately read on a calibrated scale. The Abbé is a direct reading instrument with a range of about 1.3 to 1.7. Special scales can be provided so that the instrument may be used as a direct butyrorefractometer or a sugar refractometer.

Compensation Refractometer.—When ordinary white light is used the dispersion gives a border line between refracted and reflected light which is composed of the prismatic colors. It is necessary to use a compensator to eliminate this prismatic border line and produce a sharp line of demarcation. Without this, it is impossible to use this line for proper adjustment of the instrument.

---

[1] C. A. Browne and F. W. Zerban, *Physical and Chemical Methods of Sugar Analysis*. Wiley, New York, 1941.

[2] A. E. Leach, *Food Inspection and Analysis*. Revised by A. L. Winton. Wiley, New York, 1920.

Ordinary daylight may be used for illumination of the refractometer. The instrument should not be placed in direct sunlight. Since daylight is of variable intensity, artificial light is preferable. Special illuminating devices are furnished by manufacturers. The modern high-intensity electric sodium vapor lamp is finding increasing application in refractometry. It eliminates the necessity of a compensator to correct for the dispersion of white light. This lamp is also preferable for refractometers with compensators since a sharp dividing line is obtained even with dark-colored products.

Immersion Refractometer.—Another type is the immersion or dipping refractometer. This is a limited-range instrument with correspondingly higher precision. To cover the entire range of refractive index, interchangeable prisms are provided and as many as 6 to 10 may be required. An arbitrary scale is ordinarily mounted in the eyepiece. A compensating prism is used. The temperature of the sample into which the prism is dipped should be controlled or a temperature correction should be obtained from tables. Where limited amounts of sample are available, a special cup may be used containing an auxiliary prism. The immersion refractometer is suited, for instance, to the examination of milk serum to detect added water. It may also be used for determination of purity of liquids and for the standardization of a wide variety of solutions.

Zeiss Refractometer.—Another special type is the Zeiss sugar refractometer. Improved recent designs are especially adapted for use in the sugar industry although they may be applied to other products, including those oils whose refractive index does not exceed 1.54. This instrument carries two scales, both within the field of vision, which is an advantage over the Abbé instrument. The refractive index scale covers the range from 1.33 to 1.54. It carries the third decimal place and the fourth may be estimated. The second scale is calibrated to indicate directly the percentage of sugar by weight.

Goerz Refractometer.—The Goerz sugar refractometer can be used only for sugar products since it has a fixed compensator whose dispersion is equal to the average dispersion of sugar solutions.

Special Refractometers.—There are other types of special refractometers for sugar use, some without a color compensator and employing a sodium vapor lamp such as the Bausch and Lomb instrument. There are also less precise instruments for more approximate determination of sugar, including the projection refractometer, the Zeiss, and the Bausch and Lomb hand refractometer.

Another instrument useful for specific purposes is the interferometer. This determines the difference in refraction between two liquids or gases. It is applicable to a study of fats and oils.

Selection of a Refractometer.—The selection of a refractometer must be governed by the requirements. For general purposes with products other than sugars and including substances of high refractive index, the Abbé instrument is the most generally useful. For refractive indices below 1.54, the Zeiss sugar refractometer is generally applicable and much more rapid in operation. For work on sugars exclusively, the Goerz instrument meets the requirements. The immersion refractometer should be used for greater accuracy. It requires more careful temperature control. Highest accuracy is offered by the Bausch and Lomb precision instrument. Recent developments in refractometers and their applications are discussed by Müller.[3]

(e) *Application*

Sugars.—The general applicability of the refractometer in working with sugars in solution depends upon the fact that all sugars of equal concentration have nearly the same index of refraction. Such variations as exist are discussed by Browne and Zerban. Tables showing the concentration of pure sugar solutions in relation to refractive index may be found in many handbooks, texts, and books on analytical chemistry. These are frequently based on a temperature of 20° C., but special tables for use in the tropics are based on a temperature of 28° C. Temperature correction tables and factors are also useful.

Fats and Oils.—Since different fats and oils have a characteristic refractive index, the refractometer is useful for identifying and determining the purity of this class of materials. A special butyrorefractometer is designed for this purpose. It has a scale graduated in divisions from 1 to 100 covering a variation of refractive indices from 1.4220 to 1.4895. It is useful for butterfat, and other fats and oils within this range. Conversion from scale readings to refractive indices may be made from tables. Although this instrument is intended primarily for the examination of butter and lard, it may be used for most general purposes for which the Abbé is used unless widely varying substances such as waxes and essential oils are to be studied. The refractive index of fats and oils may be found in standard texts on foods, including those earlier cited and also in the standard works on fats and oils,[4] and in the *International Critical Tables.*

Total Solids.—Refractometers have been used for the determination of total solids in solution, particularly with sugar products, since solutions of all sugars of equal concentration have nearly the same index of refraction. While this may be reasonably accurate for determination of the total amount of sugar in pure solutions, it does not necessarily agree with determination of solids by other methods. With molasses it has been found that the refractometer, depending on the method, gives $^1/_4$ to 1% higher results than the direct method. The Brix hydrometer gave results which are about 2% higher. The refractometer indicates only the percentage of dissolved solids. The refractometer appears somewhat more accurate for this purpose than the Brix hydrometer. The specific gravity and refractive index may be determined with greater precision but the results may differ somewhat from the true dry substance. This is influenced by the nature and quantity of the known sucrose constituents.

Factory Control.—Other uses of the refractometer include the determination of fine sugar crystals in molasses by determining the refractive index before and after dilution of the sample. Both refractive index and density are used as methods of factory control in the manufacture of tomato products, based on the relation between these measurements and the per cent of total solids. Tables showing the relation of these are published by Bigelow, Smith, and Greenleaf.[5] Cruess[6] discusses the use of these physical chemical methods. The amount of salt, sugar, and acetic acid used in the formula in such products will markedly affect the relation between the refractive index and total solids. In some cases it may be necessary to revise the values in the tables when formulas deviating decidedly from the ordinary are used by the manufacturer. The utilization of such methods of control may be of direct financial value to the manufacturer, since a concentration of the product beyond that required by contract may mean a material loss to the manufacturer. Such control may mean a direct increase in the value of the product because of improved quality.

---

[3] R. H. Müller, *Ind. Eng. Chem., Anal. Ed.*, **13**, 667 (1941).

[4] J. Lewkowitsch, *Chemical Technology and Analysis of Oils, Fats, and Waxes.* Macmillan, London, 1921.

A rapid refractive method for the determination of total fat in chocolate using either the Abbé or butyro instrument has been described by Stanley[7] for factory control purposes. The immersion refractometer may be used to determine and control the concentration of standard solutions and other laboratory reagents used in analytical work.

3. Optical Rotation

Optical rotation is a property of many substances, for instance, liquid turpentine, tartaric acid, and sugars, which is of particular importance from a food standpoint. This is a property found in liquids, or in solutions of the optically active materials, and also in certain materials in the crystalline state such as quartz. Light is radiant energy which has a wave length affecting the human eye. Vibrations of these waves take place in all directions, each in a plane perpendicular to the path of the beam of light. All of these waves do not penetrate all transparent materials. Tourmaline transmits light by allowing only those waves vibrating in one definite plane to pass. The light which does pass the crystal is called plane polarized light or simply polarized light. Optically active substances such as sugars have the power to rotate the plane of this polarized light. Nonoptically active substances do not affect the polarized light in this way.

(a) *Polarimeter*

A polarimeter is an instrument used to measure the amount of rotation or twisting brought about by an optically active substance. This may be illustrated by placing two books end to end. If they are lying flat on the table, a ruler may be shoved through the two books between the leaves. If, however, one of the books is rotated, it is no longer possible to insert the ruler in the two books without bending or twisting the ruler. A polarimeter contains two prisms of tourmaline, known as Nicol prisms, which are mounted at two ends of a closed tube. These are mounted in such a way that, with the polarimeter set at zero, plane polarized light passes directly through both prisms. The "leaves" of the prisms are in the same plane. One Nicol prism is so mounted that it may be rotated and the degrees through which it is rotated measured on a scale. When this prism is rotated, light entering the instrument through one prism does not pass through the second prism, and the field viewed through the eyepiece is dark. If a solution of an optically active substance such as sugar is placed in a tube and inserted into the instrument between the two Nicol prisms when the instrument is set at zero, light will also fail to pass through the second prism into the eye of the observer. If the one prism is rotated, however, a point will be reached where light will pass through the second prism and can be seen by the observer. In passing through the solution of the optically active substance, the beam of polarized light has been rotated a definite amount and by rotating the one Nicol prism a point is eventually reached where the rotation due to the optically active substance in the tube is matched or compensated for by rotation of the one prism. The amount of rotation shown by the optically active substance can then be determined by reading the number of degrees of rotation through which the moving Nicol prism has been rotated. An accurate scale is attached to the rotating prism so that the exact degree of rotation shown by the optically active substance may be read directly from the scale. D^+-Glucose, one of the reducing sugars forming a part of the molecule of ordinary sugar, rotates the plane of polarized light to the right 52.7 degrees. The optical rotation of a substance is usually expressed in terms of the specific rotation which is defined as the number of degrees of angular rotation produced in a 1-decimeter tube containing a solution of 1 g. of substance in 1 ml. volume.

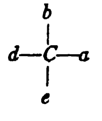
---

[5] W. D. Bigelow, H. R. Smith, and C. A. Greenleaf, Natl. Canners' Assoc., *Bull.* 27-L (1934).

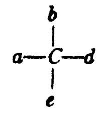
[6] W. V. Cruess, *Commercial Fruit and Vegetable Products*. 2nd ed., McGraw-Hill, New York, 1938, p. 400.

[7] J. Stanley, *Ind. Eng. Chem., Anal. Ed.*, 9, 132 (1937).

Optical activity of an organic compound is ascribed to the fact that optically active substances contain carbon atoms to which four different chemical groups are attached.



$$\begin{array}{c} b \\ | \\ d\text{—}C\text{—}a \\ | \\ e \end{array}$$



$$\begin{array}{c} b \\ | \\ a\text{—}C\text{—}d \\ | \\ e \end{array}$$

These two substances are mirror images of each other and are structurally different. Some substances rotate polarized light to the right while others rotate the light to the left. Other substances such as tartaric acid exist in two forms, one of which rotates polarized light to the left while the other rotates it to the right. A mixture of these two in proper proportions does not rotate polarized light in either direction. This is a racemic mixture. Other compounds have both a dextrorotatory group and also a levorotatory group within the molecule. The rotations in the molecule of these two groups neutralize each other so that the compound brings about no rotation of polarized light. This is called a *meso*-form. Thus we may have a *d*-form, or an *l*-form which rotates the polarized light right or left, a racemic form which is a mixture of the first two, and a *meso*-form both of which do not rotate the light. Naturally occurring tartaric acid is the *d*- or dextrorotatory form.

A large amount of work has been done on the theory and application of polarimetric measurements. This is discussed at length by Browne and Zerban.[8] Polarimetric measurements are made both for the identification of sugars and for analytical purposes. In making measurements of optical rotation, it is desirable to use monochromatic light, that is, light of a single wave length. A sodium light has been used, since this light is largely that of the *d*-sodium line in the yellow part of the spectrum. The mercury vapor lamp, however, is now quite generally used.

Saccharimeter.—The instrument commonly used in sugar analysis is a saccharimeter, rather than a polarimeter. The polarimeter is designed for the examination of all optically active substances. Saccharimeters are designed principally for polarizing sugars and the scale used is graduated according to percentage of sugar rather than according to degrees of angular rotation. The theory of saccharimeters is also discussed at length by Browne and Zerban. Saccharimeters of various types are available for different uses.

(*b*) *Factors Affecting Optical Rotation*

Various factors affect optical rotation, some of which need to be carefully controlled. Browne and Zerban discuss the effect of temperature, solvent, concentration, kind of light used, and accompanying substances, such as mineral impurities, acids, and foreign optically active substances. These authors also discuss practical methods of polarizing and analytical methods for use with various sugar products. Need for methods of clarification before polarization are discussed. Reference is made to Browne and Zerban for detailed discussion of the various phases of this complex problem, most of which cannot even be mentioned in this brief discussion.

Extensive tables are available in the literature giving the specific rotation of a large number of substances. Tables are also available in a convenient form for definite analytical applications. Polarimetric methods are also applicable to essential oils and alkaloids. Müller[9] illustrates the most recent type of precision polarimeters and double quartz wedge saccharimeters.

4. Viscosity and Plasticity

One interesting illustration of the utilization of physical chemical measurements in the food industry is the application of the determination of viscosity and plasticity to a measurement of the properties and quality of several foods. Viscosity and the influence of other factors such as *p*H on the viscosity of food colloidal systems have been studied somewhat extensively.

Viscosity has been defined as the internal friction of a liquid, the resistance of the system to flow or shear. Viscosity is measured in terms of poise, after Poiseuille, who devised methods for its measurement. Viscosity is thus expressed in absolute terms rather than in relation to a reference liquid. Standard solutions for the calibration of viscosimeters are used, however, depending on accurate calibration of these liquids by the Bureau of Standards. The poise is expressed in terms of the force which is exerted on a unit area in order to produce a given rate of flow. This may be illustrated by a simple diagram such as Figure 5 in which the viscosity is illustrated by the line *AB*, which represents a truly viscous system. Line *CD* represents the related property of plasticity or plastic flow. Figure 5 indicates that a certain amount of force is required to start the flow of the plastic substance and this is represented by *AC*. This amount of force is designated as the yield value. Plasticity is frequently considered to be more a property of solids than of liquids. The theory and practice of fluidity and plasticity are discussed at length by Bingham.[10]

---

[8] C. A. Browne and F. W. Zerban, *Physical and Chemical Methods of Sugar Analysis*. Wiley, New York, 1941.

[9] R. H. Müller, *Ind. Eng. Chem., Anal. Ed.*, 13, 667 (1941).

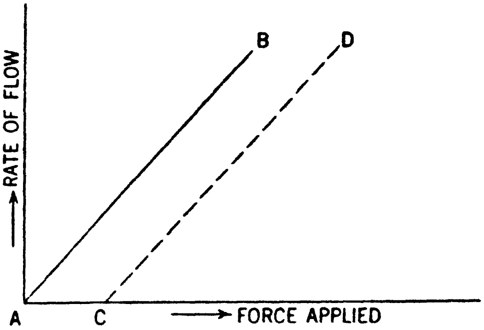


Fig. 5.—Relation between viscosity and plasticity. *AC* is the "yield value."

Colloidal systems show a wide range in viscosity and plasticity.

(a) *Factors Affecting Viscosity*

In general, the viscosity of colloidal systems decreases with increase in temperature. The viscosity of water shows a large change in raising the temperature from 0 to 100°. At 0° it has a viscosity of 1.7921 centipoises, while at 100° the viscosity is 0.2838 centipoise. The effect of temperature on viscosity is due partly to the effect of temperature on the dispersion medium, but also to the effect on solvation.

Sharp and Gortner[11] summarize certain of the literature dealing with the viscosity of colloidal systems. A number of factors in addition to temperature affect the viscosity of colloidal systems, including concentration, degree of dispersion, electrical charge, previous thermal treatment and previous mechanical treatment, presence of other lyophilic colloids, the age of such sols, and the presence of both electrolytes and nonelectrolytes. The plasticity or viscosity of colloidal systems may vary with the time or age. While the viscosity of a system is expressed in terms of the poise or centipoise, the plasticity is expressed in terms of consistency or its reciprocal mobility. This has been discussed by Kelly.[12]

---

[10] E. C. Bingham, *Fluidity and Plasticity*. McGraw-Hill, New York, 1922.

[11] P. F. Sharp and R. A. Gortner, Minnesota Agr. Expt. Sta., *Tech. Bull.* 19 (1923).

(*b*) *Measurement of Viscosity*

Ostwald Viscosity Pipet.—A variety of types of instruments have been developed for the measurement of viscosity and plasticity. One of the simplest of these is the Ostwald viscosity pipet, which has long been familiar. The results have frequently been expressed in terms of viscosity in relation to the viscosity of water. Another familiar type is the Saybolt viscosimeter, which has become a standard instrument for the determination of the viscosity of oils. These devices depend on the timing of the flow of a constant volume of liquid at a specified temperature.

Torsion Viscosimeter.—Another type is the torsion viscosimeter,[13] which has shown particular adaptability to rapid determination of the viscosity of industrial materials and has been rather widely used in the study and control of flour. The torsion viscosimeter rotates the unknown liquid in a cup at a constant rate of speed. A cylinder is suspended in the liquid by means of a torsion wire. The friction of the rotating liquid on the cylinder immersed in it twists the torsion wire and the torque of the wire measured in angular degrees can be related to the viscosity of the liquid.

Falling Ball Viscosimeter.—Falling ball viscosimeters have recently been used and depend upon the rate of fall of a sphere through a column of the liquid under investigation. This type may be made to cover a wide range of viscosity with a very high precision.

Capillary Tube Plastometer.—Plasticity has been measured by the capillary tube plastometer described by Kelly,[12] a simplified form of which has been described by St. John.[14] This has been applied to measurement of mobility of such foods as egg albumen and flour suspensions.

Power Consumption Plastometer.—Another type of plasticity technique involves the attachment of a micro watt-hour meter to a small dough-mixing machine to determine the plasticity of doughs in the process of bread making by the measurement of the total power necessary to drive the mixer 100 revolutions. This is described by Bailey.[15] A somewhat similar principle is employed by the Brabender[16] farinograph, which has been rather widely used in cereal chemistry. This may be considered as a recording plastometer. It draws a continuous graph throughout a fixed period of time. Characteristic curves are obtained with different types of flours, showing a rather marked difference, for instance, between a high-grade northern spring wheat flour and a typical pastry flour.

Dough structure is characterized by its ability to absorb more or less water. The farinograph is designed to measure and record this absorption consistency and also the stability and elasticity of the dough. Results obtained by this instrument, when properly correlated with fermentation-rate data, assist in predicting baking qualities of the flour and indicate to the expert the appropriate fermentation, kneading, and baking time to secure the best results. It is also of advantage in blending strong and weak flours.

---

[12] W. J. Kelly, *Colloid Symposium Monograph*, **3**, 303 (1925).

[13] W. H. Herschel, *Ind. Eng. Chem.*, **12**, 282 (1920).

[14] J. L. St. John, *Ind. Eng. Chem.*, **19**, 1348 (1927).

[15] C. H. Bailey, *J. Rheol.*, **1**, 429 (1930).

[16] Emil Munz and C. W. Brabender, *Cereal Chem.*, **17**, 79 (1940).

Chopin[17] (1921) devised an extensimeter using a manometer which recorded the force required to extend the surface of a thin sheet of dough which was utilized in studies of wheat flour dough. Müller[18] has discussed recent developments in this field.

Sharp and Gortner[19] made an extended study of the viscosity of wheat flour as a measurement of hydration capacity and its relation to baking strength. It was used to investigate the relation of colloidal properties of wheat proteins to the strength of flours. The difference between strong and weak flours is partially due to the colloidal state of the gluten proteins, primarily glutenin. St. John and Bailey,[20] studied the effect of the addition of dry skim milk to flour suspensions by measurement of the plasticity. St. John[21] measured the plasticity of flour-water suspensions. The mobility increased as the suspension stood, showing a time factor. Mobility, however, did not change with the age of the flour.

St. John and Green[22] measured the mobility of the thick and thin portions of egg albumen during a study of the problem of "watery whites." The time factor was evident in measuring the mobility of thick white, while the thin white did not show such a change in mobility with time. This indicated a continuous and probably natural change of thick into thin white during the storage of eggs.

Gortner[23] showed by plasticity measurements that starches derived from various botanical sources differ widely in hydration capacity. Wheat starches from different sources differ somewhat in hydration capacity.

Wilcke[24] utilized a torsion pendulum to determine the effect of individuality of the hen and the ration on the viscosity of egg albumen. The viscosity was largely an individual characteristic and was little affected by the ration.

Viscosity has an effect on heat transfer during pasteurization in preparing certain food materials, such as fruit juices. Here the viscosity and the rate of change of viscosity with change in temperature is one factor of importance. Sirups rich in pectins and gums transmit heat very much more slowly than those containing a small amount of these components. Browne and Zerban[25] discuss the use of viscosity measurements in evaluating honey. Plasticity of juice has been used to determine maturity of fruit and as an index of jellying power and sugar requirement in the production of jellies.

---

[17] C. H. Bailey and Amy M. Le Vesconte, *Cereal Chem.*, **1**, 38 (1924).

[18] R. H. Müller, *Ind. Eng. Chem.*, *Anal. Ed.*, **12**, 571 (1940).

[19] P. F. Sharp and R. A. Gortner, Minnesota Agr. Expt. Sta., *Tech Bull.* **19** (1923)

[20] J. L. St. John and C. H. Bailey, *Cereal Chem.*, **6**, 140 (1929).

[21] J. L. St. John, *Cereal Chem.*, **6**, 400 (1929).

[22] J. L. St. John and E. L. Green, *J. Rheol.*, **1**, 484 (1930).

[23] R. A. Gortner, *Cereal Chem.*, **10**, 298 (1933).

[24] H. L. Wilcke, *Iowa State Coll. Research Bull.* **194**, 77 (1936).

[25] C. A. Browne and F. W. Zerban, *Physical and Chemical Methods of Sugar Analysis.* Wiley, New York, 1941, p. 135.

5. Surface and Interfacial Tension

A liquid behaves as if there were a "skin" drawn over the surface which differs in physical properties from the bulk of the liquid beneath the surface. This surface layer is more or less rigid and constitutes the phenomenon which is called surface tension. The molecules in this surface layer are largely oriented in a particular direction, while in the body of the liquid they are distributed at random. Surface tension has also been defined as the force that impels a liquid to assume the shape presenting the smallest possible surface area. This shape is that of a sphere.

The molecules in a liquid have a definite attraction for one another. This sphere of attraction extends for only a short distance and is greatest at a distance of one or two molecular diameters. In the body of the liquid this force is exerted equally in all directions. Each molecule is completely surrounded by others which accounts for the equal attraction in all directions. Molecules at or near the surface are not completely surrounded by other molecules of the liquid exerting an attractive force. This tends to draw the surface molecules more firmly toward the body of the liquid, resulting in a molecular cohesion and orientation and a greater concentration of molecules per unit volume in the surface layer. This results in the property known as surface tension. The downward and also lateral pull of the molecules in the surface film is the force or tension which we measure, and designate as surface tension.

The term surface tension is used to refer to the phenomenon existing at a liquid-vapor interface. A similar tension, termed interfacial tension, exists at a liquid-liquid or a solid-liquid interface. The tension at a liquid-vapor and a liquid-liquid interface may be measured but a satisfactory method has not been devised for accurately measuring the tension at a solid-liquid interface.

(a) *Measurement of Surface Tension*

Capillary Tube Method.—Surface tension may be measured by the capillary tube method. The total lifting force in the capillary depends upon the radius of the capillary and is proportional to the height of the column, the density of the liquid, and the gravitational force. Surface tension equals $rhdg/2$.

Stalagmometer.—Surface tension may also be measured by the stalagmometer, frequently called the "drop weight," method which is easy of manipulation. It consists of a calibrated capillary tube with a glass bulb holding a known volume of the liquid. It carries a carefully ground tip with a flattened edge. The apparatus is filled with the liquid to be measured, and the number of drops emerging while a definite volume is eliminated are counted. It is frequently calibrated with water and a direct comparison made.

Ring Method.—Du Noüy has stated that methods for surface tension based on capillary rise or the weight of hanging drops are unsatisfactory for measuring the surface tension of colloidal solutions, and he devised the so-called ring method for surface tension. This method determines the downward pull exerted on a ring of platinum wire which is in contact with the surface of the liquid. The apparatus is designed to measure accurately the force required to break the ring loose from the surface film. This apparatus has been modified so that it may also be used to measure interfacial tension.

(b) *Factors Affecting Surface Tension*

Surface-tension values decrease with an increase in temperature. In general the decrease is directly proportional to the temperature. Surface tension is a factor in the ease with which a liquid may be vaporized. The latent heat of vaporization of water is 540 calories at 100° C. Of this, only 40 calories are needed to overcome the vapor density and atmospheric pressure. Five hundred calories are needed to overcome the surface tension and certain other forces.

Many substances in solution depress the surface tension appreciably even at low concentration. Such substances are called surface-active agents. To this group belong many organic compounds including hydrophilic colloids such as proteins. Many other substances in solution have little or no effect on surface tension at a lower concentration and are called surface-inactive. Many of the phenomena characteristic of colloidal systems are due to changes in interfacial tension. Emulsification appears to be primarily dependent upon such changes. An efficient emulsifying agent is one which lowers the interfacial tension between two mutually insoluble phases.

In measuring surface tension, it must be recognized that substances in solution do not come to equilibrium at once. The surface tension changes as the solution stands because an adsorption equilibrium is gradually established. Finally the surface tension reaches a constant value which is termed the static surface tension. The initial and intermediate values are termed dynamic surface tension and are a function of elapsed time. Some solutions reach equilibrium in a few seconds, but hydrophilic colloids sometimes require hours. Such comparisons should be made on the basis of static surface tension.

Dissolved substances which decrease interfacial energy tend to concentrate at the liquid-liquid interface. A colloidal system can decrease its surface energy by bringing into the interfacial film substances which lower the interfacial tension. Substances which decrease surface energy tend to concentrate at a liquid-vapor interface. The phenomenon of concentrating at the interface is called adsorption. Adsorption and surface tension play an important part in foaming and in commercial practices such as decolorizing with charcoal and adsorption of war gases. Adsorption is of particular importance in the purification of organic compounds. Decolorization, using bone black or an active vegetable carbon, is a common example of selective adsorption. The valuable components of a mixture may be concentrated by adsorption and subsequently the desired substance may be eluted.

Solutions of surface-active substances, especially emulsoid colloids, foam when shaken or aerated. Foaming is caused by the adsorption of the colloids on the gas bubbles in the form of a film. The quantity of foam and its stability constitute a simple approximate measure of the emulsoid colloids present. Based on this, a method has been developed to show the approximate quantity of emulsoid colloids present in sugar products and, hence, to show the quality of the sugar.

(c) *Applications*

Colloidal materials in sugar products depress the surface tension, hence the surface tension is an approximate measure of the quantity of colloidal material. As the colloidal material increases and the purity falls, there is a progressive decrease in the surface tension below that of pure sucrose solutions of the same concentration. As the specific gravity increases, however, there is a decided decrease in surface tension which, for beet sugars, reaches a minimum at about 25° Brix. The surface tension then rises with increase in degrees Brix. Although there is a general relation between surface tension and the quantity of emulsoid colloids in sugar products, it does not give a quantitative relation in the case of mixtures. Surface-tension measurements are, however, a valuable means of judging the general quality of sugars, especially of high-grade sugar. Caution should be used in interpreting the results obtained since very small traces of vegetable or mineral oils or other contaminants may be misleading and surface-tension measurements cannot be used for routine factory control.

An excellent summary of surface tension and its relation to biocolloidal systems is found in Gortner.[26] A more complete discussion of the theory of surface tension is contained in the text by Willows and Hatschek.[27] A discussion of surface tension in relation to sugar chemistry is found in Browne and Zerban.[28]

II. HYDROGEN-ION ACTIVITY

A physical chemical measurement which has aroused wide interest and has been extensively used in both research and control work in food and other industries is the measurement of the actual hydrogen-ion concentration.

1. Concentration and Activity

In earlier research and control work, acidity was measured by titration, a method which still has extensive use. While this method gives the total amount of acid or alkali present, it does not show whether the acid involved is strong or weak. It does not demonstrate the relative activity of the acid present. Two different solutions might contain sulfuric and citric acids both requiring the same amount of standard sodium hydroxide for neutralization. Equal quantities of these two would, however, behave differently in some respects, such as their power to invert cane sugar and their effect on fermentation and enzymatic activity.

---

[26] R. A. Gortner, *Cereal Chem.*, 10, 298 (1933)

[27] R. S. Willows and E. Hatschek, *Surface Tension and Surface Energy.* 3rd ed., Churchill, London. 1923.

[28] C. A. Browne and F. W. Zerban, *Physical and Chemical Methods of Sugar Analysis.* Wiley, New York 1941.

To understand what makes a substance acid or alkaline, one of the simplest of chemical compounds, water, written HOH rather than H_2O, may be used as an illustration. Water dissociates according to the following simple equation:

$$HOH \rightarrow H^+ + OH^-$$

Not only water, but all substances which are acid contain hydrogen in the form of H^+ ions. Such common acids as hydrochloric, sulfuric, and acetic all contain hydrogen which on dissociation in solution forms positive hydrogen ions.

$$HCl \rightarrow H^+ + Cl^-$$
$$H_2SO_4 \rightarrow 2H^+ + SO_4^{--}$$
$$CH_3COOH \rightarrow H^+ + CH_3COO^-$$

Common alkalies such as sodium hydroxide and ammonium hydroxide contain the OH^- ion and dissociate as follows:

$$NaOH \rightarrow Na^+ + OH^-$$
$$NH_4OH \rightarrow NH_4^+ + OH^-$$

A substance which is acid contains more hydrogen ions (H^+) than hydroxyl ions (OH^-). On the other hand, substances which are alkaline contain an excess of hydroxyl ions. The addition of a gram of HCl or a gram of acetic acid to a liter of water may be confirmed by titration in the ordinary way. The same is also true of chemically equivalent amounts, that is, in these two cases 35.5 g. of HCl and 60 g. of acetic acid per liter. These are chemically equivalent amounts. It should be definitely noted that they do not have equal effects as an acid. The hydrochloric acid has a "stronger" acid effect than the acetic acid. This is the result of the fact that these acids differ in the proportion in which they split up or dissociate in solution to form hydrogen ions. A large part, perhaps all, of the hydrochloric acid dissociates to form hydrogen ions, while a comparatively small proportion of the acetic acid dissociates, possibly 1%. Thus, the concentration of hydrogen ions per unit volume (liter) is greater in the hydrochloric solution than in the acetic acid solution when each contains one equivalent weight.

According to modern conception the term hydrogen-ion concentration refers to the "apparent" hydrogen-ion concentration, that is, the hydrogen-ion activity. The methods employed for measurement in reality measure the hydrogen-ion activity. Since it is the hydrogen or hydroxyl ion that gives acid or alkaline properties to a solution, the measurement of the hydrogen-ion concentration (activity) gives a more accurate indication of its acid or alkaline properties than does titration which determines the total amount present in the solution.

2. Method of Expression—*p*H

The concentration of hydrogen or hydroxyl ions may be expressed in terms of grams per liter (or moles per liter) but, as will be noted from Table 1, this would be a large unwieldy figure. A method of expressing this concentration in terms of *p*H was developed by Sørenson. The theory underlying this and the methods of calculation may be obtained from the references cited. On this *p*H scale, the neutral point is 7.00. This is the point where the concentration of hydrogen ions and hydroxyl ions is equal. In solutions where the hydrogen-ion concentration is larger, it is expressed by a lower *p*H value, ranging down to a *p*H of 1.0 or less. On the other hand, an increase of alkalinity (lower hydrogen-ion concentration but higher hydroxyl-ion concentration) is expressed by an increase in value for *p*H. Alkaline values of *p*H extend from 7.0 to 13.0 or 14.0. The numerals representing *p*H are not in direct arithmetical proportion to the relative hydrogen-ion concentration, since the *p*H scale is a logarithmic scale. Thus the concentration of hydrogen ions represented by *p*H 3 and *p*H 4, for instance, do not indicate a ratio of concentration of 3 to 4, or of 2 to 1, but the concentration of hydrogen ions at *p*H 3 is 10 times the concentration at *p*H 4. Similar relations hold throughout the scale.

TABLE 1

*p*H COMPARISONS AND VALUES FOR FAMILIAR SUBSTANCES[a]

Grams of hydrogen ions per liter of solution	No. of times acidity or alkalinity exceeds that of pure water (*p*H 7.0)	*p*H	*p*H of familiar substances
	10,000,000 ↑	↑ 0	
1/10	1,000,000	1.0	— 0.1 *N* Hydrochloric acid
1/100	100,000	2.0	— Human gastric contents Vinegar 0.1 *N* Acetic acid
1/1,000	10,000 acidity	Increasing acidity 3.0	— Apple juice
1/10,000	1,000	4.0	— Methyl orange changes color Grapes
1/100,000	100	5.0	— Molasses 0.1 *N* Boric acid Bread
1/1,000,000	10 ↓	6.0	— Milk Flour Fish
1/10,000,000	1 (pure water)	Neutral 7.0	— Pure water Human blood plasma Saliva
1/100,000,000	10 ↑	8.0	— Baking soda Sea water
1/1,000,000,000	100	9.0	— Phenolphthalein changes color
1/10,000,000,000	1,000 alkalinity	Increasing alkalinity 10.0	— Soap Milk of magnesia
1/100,000,000,000	10,000	11.0	— Washing soda Trisodium phosphate
1/1,000,000,000,000	100,000	12.0	— Lime water
1/10,000,000,000,000	1,000,000	13.0	— 0.1 *N* Sodium hydroxide
	10,000,000 ↓	↓ 14.0	

[a] J. L. St. John, *Food Industries*, 13, No. 12, 66 (1941).

The *p*H of pure water is 7.0. This is true only of specially purified electrolytic water. Ordinary distilled water is somewhat more acid than this and when in equilibrium with air, the *p*H is 5.8 or 6. The *p*H of ordinary tap water may vary over a wider range extending into the alkaline values above 7, depending upon the nature and quantity of dissolved salts.

3. Normality and *p*H

The term normality is used to describe the relative concentration of acids and bases. A 0.1 *N* solution of HCl, which is a strong acid, has roughly a *p*H of 1.0. This is not true, however, for a weak acid such as acetic acid, since a 0.1 *N* solution of this acid has a *p*H of 2.9, while a 0.1 *N* solution of boric acid has a *p*H of 5.2. Other values are indicated in Table 2. These acids all have the same concentration of total

TABLE 2

*p*H VALUES OF VARIOUS ACIDS, BASES, AND COMMON SUBSTANCES[a]

0.1 *N* Acids and Bases

Acids	*p*H Value	Bases	*p*H Value
Hydrochloric acid	1.0	Sodium bicarbonate	8.4
Sulfuric acid	1.2	Borax	9.2
Phosphoric acid	1.5	Ammonia	11.1
Sulfurous acid	1.5	Sodium carbonate	11.6
Acetic acid	2.9	Trisodium phosphate	12.0
Alum	3.2	Sodium metasilicate	12.2
Carbonic acid	3.8	Lime (saturated)	12.3
Boric acid	5.2	Sodium hydroxide	13.0

Common Substances

Apples	2.9-3.3	Limes	1.8-2.0
Apricots (dried)	3.6-4.0	Magnesia, milk of	10.5
Asparagus	5.4-5.7	Milk, cows	6.4-6.8
Beans	5.0-6.0	Molasses	5.0-5.4
Beers	4.0-5.0	Olives	3.6-3.8
Beets	4.9-5.6	Oranges	3.0-4.0
Blackberries	3.2-3.6	Peas	5.8-6.4
Bread, white	5.0-6.0	Peaches	3.4-3.6
Cabbage	5.2-5.4	Pears	3.6-4.0
Carrots	4.9-5.2	Pickles, dill	3.2-3.5
Cherries	3.2-4.1	Pickles, sour	3.0-3.5
Cider	2.9-3.3	Pimento	4.7-5.2
Corn	6.0-6.5	Plums	2.8-3.0
Crackers	7.0-8.5	Pumpkin	4.8-5.2
Dates	6.2-6.4	Raspberries	3.2-3.7
Flour, wheat	6.0-6.5	Rhubarb	3.1-3.2
Ginger ale	2.0-4.0	Salmon	6.1-6.3
Gooseberries	2.8-3.1	Sauerkraut	3.4-3.6
Grapefruit	3.0-3.3	Shrimp	6.8-7.0
Grapes	3.5-4.5	Spinach	5.1-5.7
Hominy (lye)	6.9-7.9	Squash	5.0-5.3
Human blood plasma	7.3-7.5	Strawberries	3.1-3.5
Human duodenal contents	4.8-8.2	Sweet potatoes	5.3-5.6
Human feces	4.6-8.4	Tomatoes	4.1-4.4
Human gastric contents	1.0-3.0	Tuna	5.9-6.1
Human milk	6.6-7.6	Turnips	5.2-5.5
Human saliva	6.0-7.6	Vinegar	2.4-3.4
Human spinal fluid	7.3-7.5	Water, distilled (equil. with air)	5.8
Human urine	4.8-8.4	Water, distilled (CO_2 free)	6.8-7.0
Jams, fruit	3.5-4.0	Water, mineral	6.2-9.4
Jellies, fruit	3.0-3.5	Water, sea	8.0-8.4
Lemons	2.2-2.4	Wines	2.8-3.8

[a] J. L. St. John, *Food Industries*, 13, No. 12, 67 (1941).

acid, but the hydrogen-ion concentration or *p*H varies. The acids are, therefore, said to vary in strength. The terms concentration and strength should not be confused, although they have been interchangeably used rather widely. Strength and weakness refer to the relative activity which, in the case of acids and bases, is expressed in terms of *p*H or hydrogen-ion concentration. Concentrated and dilute are terms which refer to the total quantity present. A solution containing a large total quantity of acid or alkali is concentrated, while a solution containing a small total quantity is dilute.

4. Buffer Solutions

Pure water is completely unbuffered. It contains nothing which will affect the change of hydrogen-ion concentration if acids or bases or any substances carrying hydrogen or hydroxyl ions are added to it. The change in *p*H of pure water will be proportional to the quantity of hydrogen ions added to it.

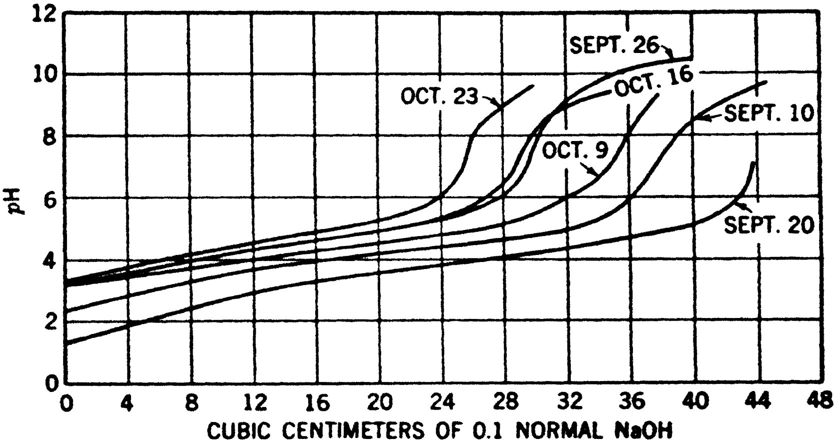


Fig. 6.—Titration curves showing natural buffer value of apple juice.

Solutions may be prepared which are not buffered. Most or all natural biological solutions, however, or those prepared from food are buffered. They contain salts or other materials of various types which inhibit the change of hydrogen-ion concentration of the solution. The change in *p*H is not proportional to the quantity of hydrogen or hydroxyl ions added. This is illustrated by the titration curves in Figure 6 published by St. John and Morris,[29] where apple juice was titrated with 0.1 *N* sodium hydroxide. At different harvest dates, the *p*H of the original juice varied between 1.32 and 3.35. As standard sodium hydroxide was added the curves showing the change in *p*H were essentially straight lines until a *p*H of approximately 5.0 was reached. Thereafter, the change in *p*H due to the addition of a given quantity of sodium hydroxide increased much more rapidly. Below a *p*H of 5.0 it required a fairly large quantity of sodium hydroxide solution to effect a material change in *p*H. The apple juice was naturally buffered.

---

[29] J. L. St. John and O. M. Morris, *J. Agr. Research*, 39, 623 (1929).

The addition of 1 ml. of 0.01 *N* HCl to a liter of pure water changes the *p*H to approximately 5.0 since there is no buffer present to retard the effect of the added acid. If, however, the same quantity of hydrochloric acid is added to a solution containing sodium acetate and acetic acid, the change in *p*H value is very small. The sodium acetate and acetic acid repress the formation of hydrogen and chloride ions from the hydrochloric acid or, in other words, they have acted as buffers. The hydrochloric acid reacts with the sodium acetate to form acetic acid, and the total concentration of the latter is slightly increased. Since acetic acid is a comparatively weak acid (slightly dissociated) the final result of the addition of hydrochloric acid is a comparatively small increase in the concentration of hydrogen ions, rather than the large increase which would have been the case if hydrochloric acid alone (highly dissociated) had been added to the pure water.

Buffers may be defined as substances which, by their presence in solution, increase the amount of acid or alkali that must be added to cause a unit change in *p*H. They may also be defined as those substances which prevent sudden or great changes in hydrogen-ion concentration when strong acids or bases are added to the system. The most efficient buffers are mixtures of weak acids or bases with their corresponding salts.

Many other materials act as buffers, including proteins, citric acid and citrates, carbonic acid and carbonates, phosphates, and others. These may exert buffer action over different parts of the *p*H scale. Salts of strong acids or bases do not buffer a solution except perhaps in very strongly acid or alkaline solutions. A solution of sodium chloride or sodium sulfate may therefore be practically as completely unbuffered as distilled water. The strength of the buffer action depends on the total concentration of the acid and salt present, the ratio of salt to acid, and other factors. Buffer strength may vary from highly buffered to completely unbuffered materials. The buffer strength of unknown solutions can be determined by making titration curves similar to those in Figure 6.

5. Methods of Determination

The determination of the *p*H of highly buffered solutions requires no special precaution since the *p*H is not easily changed. Great care, however, must be used in determining the *p*H of slightly buffered or unbuffered materials, such as pure sugar liquors since *p*H is readily altered. The buffering action of milk is a maximum at *p*H 5.5. Casein is believed to be a principal factor in this buffer action. It is believed that phosphates in milk constitute the most important buffer.

(*a*) *Colorimetric Methods*

There are two general methods for the determination of the *p*H value of solutions, the colorimetric and the electrometric. In titration common indicators are used, each of which changes color at its own *p*H level. Phenolphthalein changes at about *p*H 9.0, while methyl orange changes at about 4.0. Indicators undergo changes in color as the *p*H changes over a range. These colors can be stabilized at different *p*H values by the use of buffers. Thus a series of stable color standards can be prepared and used in the colorimetric measurement of *p*H. A colorimetric comparison set can be prepared using several different indicators which will cover the entire usable range of *p*H values. To determine the *p*H of an unknown solution, a measured quantity of the proper indicator to cover the *p*H range of the unknown solution can be added to this solution and the resulting color compared to the buffered standards. These are frequently furnished in sealed comparison tubes. Comparator sets of several different types are available in convenient form for commercial control laboratories. Laboratories may also prepare their own standard buffer solutions utilizing tables available in the texts cited below, and also by Gortner.[30] By purchasing or preparing indicators covering the range desired, and preparing standard buffers based on these tables, laboratories may make their own colorimetric *p*H comparisons.

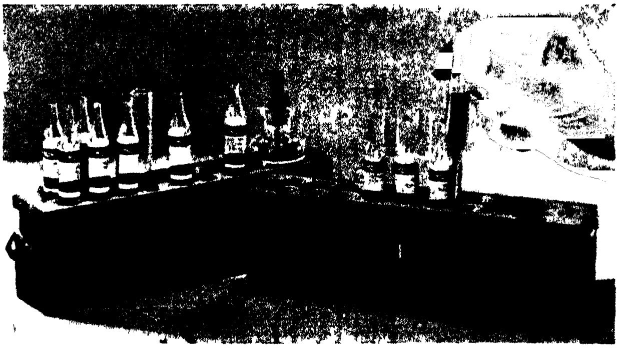


Fig 7 —Colorimetric *p*H comparator set
(Courtesy of the Van Sant, Dugdale Co , Inc)

Naturally, colorimetric procedures for the determination of *p*H are used mainly with colorless solutions or with slightly colored solutions which have been standardized. Turbid solutions also offer difficulty in using the colorimetric procedure. The *p*H of solutions forming precipitates or unusual color compounds with indicator solutions can seldom be obtained by colorimetric procedures. In general, the accuracy of colorimetric *p*H measurements is not so high as that of the electrometric procedures. In many cases the precision of the colorimetric procedure may not extend beyond 0.1 to 0.2 *p*H.

(*b*) *Electrometric Method*

The electrometric method of *p*H measurement in some of its modifications is utilized when greater preciseness is desired or when the colorimetric method is not applicable. Recent developments have greatly simplified the electrometric procedure from one which in some instances was difficult for the physical chemist to one which may be utilized rapidly and satisfactorily for routine work by some who are not well trained in chemistry. The glass electrode is widely utilized in such instruments. For a discussion of the theory of the glass electrode the reader is directed to the references at the end of this chapter. Compact portable instruments are available from several companies which may be utilized for rather precise rapid measurement of *p*H in a variety of types of solutions. Recent industrial models are convenient for use although not as accurate as research models.

---

[30] R A Gortner, *Outlines of Biochemistry* Wiley, New York, 1938.

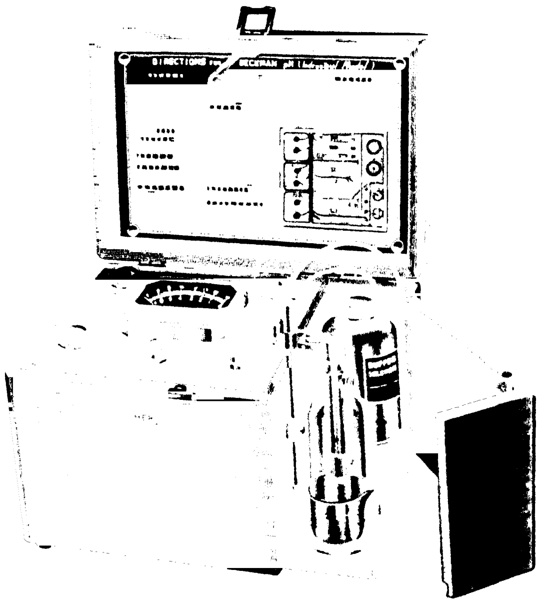


Fig 8 Electrometric *p*H meter (industrial model) (Courtesy of the Fisher Scientific Co)

The glass electrode is perhaps the most practical device for determining *p*H. Glass electrode potentials depend only on the *p*H and are not ordinarily affected by the presence of oxidizing or reducing agents, gases, chemical compounds, colloids, or suspended matter. The glass electrode does not require the use of hydrogen gas, quinhydrone, or other chemicals. Suitable reference electrodes, a liquid junction, and a voltage measuring device are necessary. Vacuum tube amplification is used. At high *p*H values where the hydrogen ion concentration is low, primarily above *p*H 9.0, the glass electrode surface responds to ions other than hydrogen ions The error introduced depends on the *p*H, temperature, and on the kind and concentration of foreign ions. Most difficulty is experienced with the sodium ion. Correction factors may be developed. There may also be slight errors in highly acid solutions below *p*H 1.4.

(*c*) *Other Systems of Measurement*

Other systems of *p*H measurement have been used, including the quinhydrone electrode which is reasonably exact up to a *p*H of about 8.5. It is, however, seriously affected by oxidizing or reducing agents in the solutions, it is subject to salt error, and is adversely affected by proteins and other electrode poisons. The results are not reliable in unbuffered samples. Metal oxide electrode combinations such as the antimony electrode may be inaccurate and subject to numerous errors.

(*d*) *Electrometric Titration*

In addition to *p*H measurements, electrometric equipment may be used to determine the end point during the titration of colored solutions where indicators cannot be used to determine the end point in the ordinary way. Titration curves similar to Figure 7 may be constructed, or the *p*H may be determined only near the desired end point. These procedures are especially applicable to the titration of such materials as fruit juices and milk.

In plant control work *p*H measurements should be under the supervision of, and should be frequently checked by, a well-trained chemist to insure that they maintain their accuracy. Such instruments and measurements should not be used for research by those untrained in chemistry who do not have an understanding of the principles and fundamentals of *p*H and its measurement.

6. Isoelectric Point

Such measurements have many applications not only in research, but also in industrial control in food-manufacturing and other plants. The properties of gelatin, for instance, depend on the *p*H. At 4.7 gelatin does not combine with either positively or negatively charged ions. This is the gelatin isoelectric point. At higher *p*H values, gelatin combines with positively charged ions such as hydrogen or metal ions, while at lower values it combines with negatively charged ions. The destruction of gelatin is least at the isoelectric point. The isoelectric point is the place where the electric charge on the molecule is neither positive nor negative. It is electrically neutral. It is a physical constant of all proteins. At the isoelectric point the characteristic properties of the proteins exhibit maximum and minimum values. At the maximum are analytical purity, turbidity, and lowering of surface tension. At the minimum are viscosity, optical rotation, stability in solution, ash content, electrical conductance, osmotic pressure, and membrane potential. Egg albumin is a protein with isoelectric point of *p*H 4.55.

7. Relation to Jelly Strength

Swelling in water, viscosity, and jelly strength depend on the *p*H, the minimum swelling occurring at 4.7 and the viscosity and jelly strength also being at minimum at or near this point. There are two points of maximum swelling, one at *p*H 2.4 and the second at 11.6. These are also maximum for viscosity and jelly strength. The properties of fruit jellies depend on the *p*H. The optimum is about 3.3 while the practical limits are 3.1 to 3.55. Above 3.55 the fruit juices may not form a jelly, while below 3.1 the jellies are weaker and syneresis may result. Consequently *p*H control is also of importance in the manufacture of jams and similar products.

8. Application

The control of *p*H is of importance in the manufacture of bread. Diastatic activity is a maximum at values ranging from 4.5 to 5.0. It has been stated that the best *p*H value for dough at time of baking is 5.0.

*p*H level is also of importance in the sterilization and keeping of foods. The lower the *p*H, that is, the higher the acid potential, the lower the degree of heat necessary for sterilization. Foods which have a low *p*H value require less processing than foods with higher *p*H values such as meat and fish. Molds grow at lower *p*H values than bacteria, and prefer a slightly acid medium. Most bacteria show preference for a slight alkalinity. Fruits are more subject to mold, while meats are more often affected by bacteria, since the fruits are the more acid.

Fermentation and enzymatic action are influenced by hydrogen-ion concentration. Often a suitable control of the *p*H of the fermentation mash promotes maximum activity of the desired enzymatic activity and inhibits the activity of undesirable organisms. This finds application in the manufacture of vinegar and in cheese. Establishment of optimum *p*H level may decrease the time of normal fermentation. The *p*H of cane-sugar liquors is carefully controlled in refining. There is an optimum *p*H for the elimination of impurities and a low *p*H which causes inversion of sucrose must be avoided, while high *p*H may cause destruction of glucose and formation of colored compounds. *p*H is closely controlled throughout every step of the sugar-manufacturing process. The *p*H of fresh eggs rapidly changes in storage. When fresh, the *p*H of egg white is about 8.0 as shown by the results of a number of more recent workers. Earlier results seemed to show a value of 7.6. This rapidly changes and frequently reaches a value of 9.0 or 9.5 in a few days.

Bridges and Mattice[31] present an extensive table of *p*H values on more than 400 representative foods involving 2100 determinations.

For theoretical and detailed consideration of hydrogen-ion activity, the reader is referred to a variety of discussions, including the books by Clark,[32] Michaelis,[33] and Kolthoff and Laitinen.[34] Good summarized discussions of the theory and practice of *p*H, glass electrodes, buffer value, and other factors are issued by companies manufacturing electrometric and colorimetric types of equipment for the determination of

---

[31] M. A. Bridges and M. R. Mattice, *Am. J. Digestive Diseases Nutrition*, **6**, 440 (1939).

[32] W. M. Clark, *The Determination of Hydrogen Ions* 3rd ed., Williams & Wilkins, Baltimore, 1928.

[33] L. Michaelis, *Hydrogen Ion Concentration. 1. Principles of the Theory.* Translated by W. A. Perlzweig. Williams & Wilkins, Baltimore, 1936.

[34] I. M. Kolthoff and H. A. Laitinen, *Potentiometric Titrations.* Wiley, New York, 1941.

*p*H. Many specialized applications of *p*H measurements will be found discussed in the literature.

III. COLLOIDAL CHEMISTRY OF FOODS

Many foods exist in a colloidal condition. Using the proper technique, any material may be brought into the colloidal state. There is no distinct dividing line between materials which may be regarded as colloidal and those which generally exist as noncolloids. Colloid phenomena deal with a state of matter and not a kind of matter. Thus the statement that a particular material is a colloid is not strictly correct. Under certain conditions a material may exist in a colloidal condition while under other conditions it is not colloidal.

The physical properties of a system will show whether or not a particular material is colloidal or crystalloidal. Even a material like sodium chloride, using proper methods and a proper dispersion medium, may be made to exist in the colloidal condition. Tannin may be dispersed in water in the colloidal state while in acetic acid it forms a crystalloidal solution.

A given compound or material cannot exist in the colloidal condition by itself. Only when properly dispersed in a second medium can it exist in the colloidal condition. A colloidal system is a heterogeneous system. Various terms are used to describe the two parts of a colloidal system. Frequently, the material which forms the basis of the system is described as the dispersion medium. It is the continuous phase. The compound or material which exists in a colloidal condition is called the disperse phase or the discontinuous phase. Colloidal systems containing a disperse phase and a dispersion medium are designated as sols. A sol may be defined as a colloidal system which has the appearance of a solution. It is fluid and although it is not homogeneous it appears to be so.

It is stated that colloid systems result whenever one material is divided into a second with a degree of subdivision coarser than molecular. In general, a material shows colloidal properties in the dispersion medium when the particles are between 1 mμ and 0.01 mμ in diameter. It should be strongly emphasized that these dimensions are arbitrary boundaries. The properties of colloidal systems do not strictly coincide with these arbitrary boundaries. There is a continual gradation in the properties from strictly crystalloidal systems to coarsely divided suspensions.

1. Classes of Colloids

Since there are three states of matter—solid, liquid, and gas—nine classes of colloidal systems would appear possible. A solid-in-solid colloidal system may be illustrated by the black diamond, which is carbon dispersed in crystalline carbon. This class is relatively unimportant from a biological standpoint. A solid-in-liquid system may be represented by sodium chloride in alcohol or by starch in water at lower temperatures. This class has been described as a suspensoid. It is a system in which the disperse phase is insoluble in a dispersion medium, and is termed a lyophobic system. The solid-in-gas class may be represented by the blue haze resulting from forest fires. The opal and pearl are representative of the liquid-in-solid class. The opal is a system of silicon dioxide and water, and the pearl is a dispersion of calcium carbonate in water. In biology, the class of liquid-in-liquid colloids is the most common. Such colloidal systems are called emulsoids. The disperse phase and the dispersion medium are mutually more or less soluble one in the other. Substances which have an affinity for the dispersion medium are called lyophilic colloids. Hydration of such colloidal materials takes place if water is the dispersion medium. This class is illustrated by gelatin in water. Gelatin swells in water and some of it disperses in the water while a material amount of the water dissolves in the gelatin. Gelatin in water is hydrated. It forms a lyophilic system. Gelatin in alcohol or benzene is a lyophobic system. A liquid-in-gas colloidal system is illustrated by fogs and mists. The form in which a gas is present in solids may frequently be debatable. Adsorption is discussed on p. 38. Typical gas-in-liquid colloidal systems result when egg white is beaten and forms a foam, and when cream is whipped. The lyophilic colloids present stabilize the resulting foam. Since gas is always considered as occurring in the molecular state, the ninth class of gas-in-gas colloidal systems is theoretically impossible.

2. Methods of Preparation

There are a variety of practical methods for the preparation of colloidal systems, only a few of which will be mentioned. They include crystallization or condensation methods and solution or dispersion methods. Peptization is undoubtedly the most important of all dispersion methods. Peptization methods fall into four general classes, including the washing out of a precipitating agent. Many organic substances such as gelatin and dextrin are peptized by the direct addition of water. Occasionally it is necessary to use heat to obtain complete dispersion. Agar and gelatin form gels in cold water, but sols in hot water. Soap acts as a peptizing agent in the removal of dirt from clothing. The use of soaps in the formation of kerosene emulsions and similar emulsions is another example of the practical application of peptizing agents.

Peptization occurs when energy is added to a system. Peptization may result from grinding. Lyophobic sols require a greater expenditure of mechanical energy than does the formation of lyophilic sols. Colloid mills are used for the preparation of industrial colloidal materials, although such mills, strictly speaking, are misnamed since they do not reduce the materials to colloidal dimensions. They are, however, important in the making of emulsions or in finely dispersing any material in a liquid. The fourth general class of peptization methods results from the formation of colloidal systems by the use of electrical energy.

3. Properties

(a) *Osmotic Pressure*

While osmotic pressure is very important in studying the properties of true solutions, it is not an important characteristic of truly colloidal systems. Although the gas laws and laws of diffusion may be considered to apply to colloidal systems, if certain assumptions are made, actually the osmotic pressure of sols such as dispersed proteins is extremely low even when the biocolloid is present in monomolecular condition. Although egg albumen is said to exist in sols in a monomolecular condition, such sols are believed to be typically colloidal. In most lyophilic systems the osmotic pressure of the sol is low, and in many lyophobic systems the osmotic pressure value may be too small to be measurable.

(*b*) *Dialysis*

Nondiffusion through a membrane has been considered a criterion of the colloidal state. This forms the basis of a method of separation of colloidal materials from crystalloidal and may be used in the purification of substances in colloidal condition by allowing salts or other crystalloidal materials to diffuse out leaving the purified colloidal material. This separation of the colloids from crystalloids is called dialysis. Many types of membranes are used in dialysis. This method is not always adaptable to a clean separation of colloid and crystalloid, since some of the smaller colloidal particles may diffuse through the semipermeable membrane. Thus, under certain conditions, an appreciable amount of egg albumen may dialyze through a membrane. Methods are available for preparing membranes with different sized pores to meet the different needs in dialyzing and purifying different colloidal materials. Colloidal sols usually pass unchanged through ordinary filter paper since the pores are approximately 2 to 5 mμ in diameter.

Ultrafiltration.—Filters have been devised with pores sufficiently fine to allow only the dispersion medium and its crystalloidal solutes to pass through. This process of separation is known as ultrafiltration. Such ultrafilters are colloidal gels so that ultrafiltration is the filtration of a sol through a gel. Commonly used membranes are collodion or gelatin. Rubber and synthetic plastic membranes have been used. Almost any degree of permeability of collodion membranes may be obtained by mixing varying proportions of glycerol and castor oil with collodion sol. Ultrafiltration may be conducted under high pressure if the membrane is adequately supported.

(*c*) *Electrodialysis*

Dialysis, which depends upon diffusion alone, is often an extremely slow process and is frequently incomplete. The removal of electrolytes from colloidal sols and gels may be hastened by dialyzing with the aid of an electric current, and the colloidal material may thus be obtained in a higher degree of purity. The electrolytes may be almost completely removed. The dialyzing process using an electric current is called electrodialysis. It is widely used as a method of rapid purification of colloidal systems from electrolyte contamination. It has been applied to the purification of such materials as cellulose and in general it offers an efficient method for the purification of lyophilic colloids. Since electrolytes are frequently adsorbed on the surface of colloidal micelles, they can only be removed from such surfaces by an impressed electrical potential.

(*d*) *Optical Properties*

Colloidal systems exhibit certain optical properties of some importance. A beam of light projected through air makes floating particles visible. Similarly, colloidal particles in a sol may become visible if a beam of light is projected into the fluid. Lyophobic colloids show intense Tyndall cones while lyophilic colloids may show a slight or no cone. The color of the cone may depend upon the size of the colloidal particles, and colors do not necessarily indicate chemical differences.

Some lyophilic systems which do not show a Tyndall cone with ordinary light do show a definite visible cone when a beam of ultraviolet light is projected into them. This phenomenon is known as fluorescence, the ultraviolet light being transformed into visible wave lengths. Proteins, sols, and gels in particular show such fluorescence.

(*e*) *Surface Tension and Interfacial Tension*

Surface tension and interfacial tension play an important part in certain colloidal phenomena. A solution may decrease its surface energy by transferring to the surface either an excess of solvent or an excess of solute, depending upon which one causes the lowering of the surface tension. A colloidal system can decrease its surface energy by bringing into the interfacial film substances which lower the interfacial tension. Substances which decrease surface energy tend to concentrate at a liquid-vapor interface. Those substances which decrease interfacial energy tend to concentrate at a liquid-solid or liquid-liquid interface.

(*f*) *Adsorption*

The phenomenon of concentration at the interface is called adsorption. A stoichiometrical chemical equation to represent adsorption cannot be written. Adsorption is an equilibrium which depends upon the concentration of the material which is being adsorbed and on the extent of the surface upon which adsorption may take place. There is some difference of opinion regarding the mechanism of adsorption and it is believed by some to be stoichiometrical. They believe, for instance, that acid-base exchange in soils and minerals and acid and alkali binding of proteins and the relationships between biocolloids and the electrolytes of biological systems are quantitative. It is probable that the facts are intermediate between a strictly stoichiometrical combination and purely physical surface adsorption.

The amount of material adsorbed is directly proportional to the specific surface of a given adsorbent. It is sometimes difficult or impossible to determine the surface area of an adsorbing solid. The reverse of this process, however, is sometimes used and the surface determined by the extent of the adsorption. The nature of the surface of a solid may determine to a large degree its behavior in acting as an adsorbent. Charcoal must be activated to become an efficient adsorbent. One of the more common methods of activation is to heat charcoal in a current of steam and later in a closed container at 700 to 800° C. The nature of the solvent is also of great importance in adsorption reactions. Adsorption is greatest from solvents which have a high surface tension. A substance may be positively adsorbed from water but only slightly or not at all from an organic solvent. Selective adsorption may be used for purification, a common example in the food industry being the decolorization of a sugar solution with bone black or an activated vegetable carbon. Great care should be exercised to determine whether the use of an adsorbent may cause a loss of an appreciable amount of a valuable component. Large losses may occur. Quantitative preliminary studies should always be made before utilizing a carbon adsorbent to prove that the valuable component is not lost in the process. Also, washing a carbon adsorption complex with an organic solvent may entirely defeat the original purification program. It may also, however, be possible to concentrate all the valuable components by adsorption with subsequent elution with the proper solvent. In general, adsorption is characterized by a positive heat of adsorption. In some cases, the amount of heat liberated is large.

(*g*) *Foaming*

In beating an aqueous solution of egg albumen into a foam, the egg protein is adsorbed at the gas-liquid interface and the adsorbed egg albumen is coagulated by the surface energy similarly to heat coagulation of egg albumen. This denatured albumen cannot be dissolved in water and the insoluble film of albumen thus formed prevents a reversal of the adsorption equilibrium. Egg protein can be removed practically quantitatively by bubbling air through a dilute solution of the albumen. Nearly all of the original egg albumen in the form of insoluble films may be filtered off after standing. Such processes are considered to be pseudo-adsorption, since there is a chemical change in the adsorbing material which inhibits a reversal of the adsorption process.

(*h*) *Removal of Water*

Many foods are hydrophilic colloids, including proteins, carbohydrates, and lipids, to which water is rather tightly bound. The removal of moisture from such foods is an empirical procedure determined by three variables—temperature, pressure, and time. The removal of water is merely a shifting of the existing equilibrium to a new equilibrium and the extent to which this equilibrium is shifted is determined by these three variables, which must be properly controlled in each individual instance to secure the proper dehydration.

4. Gels and Sols

Gels may be defined as more or less rigid colloidal systems. Gels frequently differ from lyophilic sols solely in concentration or in degree of dispersion. The equilibrium from gel to sol may in some cases be affected by a change in concentration of the disperse phase. The equilibrium may be more or less readily reversed. A suspension of starch in water may be a sol at higher temperatures which will be converted into a gel if the temperature is lowered. The viscosity and plasticity of gels may vary widely. Gels may have a low viscosity and flow under the application of slight pressure or they may be very rigid structures. Properties of such systems vary with their physical structure. Gels undoubtedly have a structure. This has been described as a "brush-heap" structure. There are a number of general methods for the formation of gels. A sol may "set" following a change in temperature, hydrogen-ion concentration, or electrolyte content. A gelatin gel will form from a gelatin sol although some time is required for the formation of a rigid gel. Gels may also be formed by dialyzing out a peptizing agent from a sol. If a solid, such as gelatin, imbibes a dispersion medium such as water a gel is formed. A gel may also be formed by the addition of a third phase to a system.

5. Imbibition

Colloids frequently show a great affinity for certain liquids and imbibition occurs against relatively large pressures which are described as imbibition pressures. In egg white this may be illustrated by the fact that water may be so closely bound by the white that it cannot be separated by freezing except at temperatures much below the freezing point of water.[35] A striking example of imbibition pressure is shown by the common cactus which retains water under extremely dry conditions. Imbibition pressure is shown by the fact that starch will swell against a pressure of 2500 atmospheres if it is heated in the presence of water.

Imbibition should not be confused with solution. Imbibition is usually accompanied by evolution of heat in contrast to solution. Hydrogen-ion concentration has a marked effect on imbibition, particularly with proteins; a minimum degree of swelling occurs in solution when the protein is at the isoelectric point. At hydrogen-ion concentrations on either side of this point there is a rapid increase in imbibition which reaches a maximum at approximately *p*H 2.5–3.0 and at *p*H 10.5. In contrast to proteins the carbohydrate gels like agar show a maximum imbibition in solutions at or near the neutral point with a decrease in imbibition capacity as the *p*H recedes from the neutral point.

(*a*) *Syneresis*

Syneresis is considered to be essentially the reverse of imbibition. The liquid which exudes from a gel during syneresis is not pure water but is a dilute solution, the composition of which is determined by that of the original gel. The "bleeding" of an agar slant, the liquid which collects around the mold of gelatin in an icebox, the separation of whey during the souring of milk or during the cheese-manufacturing process, the "sweating" of bread, "leaky" butter, the separation of a liquid from lean meat when heated, are all typical examples of syneresis.

---

[35] J. L. St. John, *J. Am. Chem. Soc.*, **53**, 4014 (1931).

(*b*) *Hysteresis*

A time factor is characteristic of all colloidal systems but is particularly noticeable in gels. Micelles may aggregate into larger units. Larger particles may grow at the expense of smaller ones, as the system ages. In biocolloids, there is a decrease in imbibition capacity with age. Older tissues, both plant and animal, are generally less highly hydrated than younger tissues. Muscle tissues have a progressively lowered imbibitional capacity as the animal becomes older. Hysteresis effects are most strikingly shown by viscosity or plasticity methods. This is illustrated by St. John and Green[36] who showed a distinct time factor in the plasticity of the thick portion of egg white. They also postulated a change of thick to thin white with time, and this may be a case of syneresis. The "aging" of a gel is characterized by a decrease in the intensity of the forces with which the dispersion medium is held. In using viscosity and plasticity methods, the past history of the colloidal system may well be the most important variable. It is essential to closely duplicate every detail of mechanical, thermal, or electric treatment, and to observe exact time intervals in order to duplicate the viscosity or plasticity of lyophilic colloidal systems. The hysteresis effect of a natural biological gel is interestingly shown by the effect of frosting on moist immature wheat. After harvesting and drying, such wheat has a much higher hydration capacity and tends to imbibe water more readily and reach a higher water content at equilibrium than unfrozen wheat harvested in a normally dry condition.

(*c*) *Hydrated Colloids*

Proteins are hydrophilic colloids and may become strongly hydrated. The large amount of water present in living organisms is probably due mainly to the affinity of proteins for water. Many of the polysaccharides of plant material are highly hydrophilic colloids and have relatively large amounts of water associated with them. The fats and lipids of living organisms exist in the form of emulsions. Fat tissue contains as much as or a larger proportion of water than fat.

The chapter on the subject of "bound water" which is associated with hydrophilic biocolloids is an extremely interesting one and the principles underlying this phenomenon may play an increasingly important role in food chemistry. Bound water has been discussed at some length by Gortner.[37]

6. Emulsions

Although the range of the particle size in emulsions generally exceeds the upper limit of the colloidal realm, emulsions may, however, be classified as a fifth group of colloidal systems formed by peptization. Many of the fat droplets in emulsions are visible in the ordinary microscopic field. A simple emulsion may be defined as a mixture of two mutually insoluble liquids. From this it is clear that there are two different types of emulsions which are possible depending upon which liquid forms the disperse phase and which one constitutes the dispersion medium. Water is almost invariably one of the phases in biological systems. We thus ordinarily have two forms of emulsions, one an oil-in-water, and the second a water-in-oil. Pure emulsions containing only one pure liquid dispersed in another pure liquid are difficult to produce. Very dilute emulsions of this type may be formed, for instance, during the distillation of volatile liquids. This sometimes causes an industrial loss and with certain essential oils the valuable component remaining in the emulsion must be recovered by extracting with a solvent or by using some means of breaking the emulsion in order to recover the oil.

---

[36] J. L. St. John and E. L. Green, *J. Rheol.*, **1**, 484 (1930).

[37] R. A. Gortner, *Outlines of Biochemistry*. Wiley, New York, 1938.

(*a*) *Stabilization*

Since such simple pure emulsions are difficult to stabilize in any practical concentration, emulsions are prepared practically by employing two principal methods. To stabilize an emulsion, it may be passed through a colloid mill or other homogenizing machine or substances may be introduced which retard the rate at which oil globules coalesce. These substances fall into three groups: (1) those which equalize the specific gravity of the two phases, (2) those which increase the viscosity such as gums, and (3) those which consist of long-chain molecules with a hydrophilic group at one end and a hydrophobic group at the other. Substances in group (3) are correctly designated emulsifying agents. Water-in-oil emulsifiers are more soluble in lipids than in water and oil-in-water emulsifiers are more soluble in water than in lipids.

Gums, such as acacia, are common stabilizers for oil and water emulsions. Acacia gum is not attacked by putrefying bacteria as are proteins. The gum disperses in water and forms a lyophilic hydrosol. In general, water-soluble emulsion stabilizers or stabilizers which form lyophilic hydrosols produce emulsions of the oil-in-water type. On the other hand, oil-soluble stabilizers which are insoluble in water produce emulsions of the water-in-oil type. Water-soluble soaps such as sodium or potassium soap are stabilizers of oil-in-water emulsions. Calcium soaps, since they are water insoluble and oil soluble, are efficient stabilizers of water-in-oil emulsions.

In general, natural emulsions, such as milk, are stabilized by proteins. A special protein stabilizes the fat droplets in milk and cream. Mayonnaise dressing is stabilized by egg-yolk proteins. A stable French dressing emulsion from olive oil and vinegar may be made by using a small amount of gelatin. The amount of the disperse phase which may be present in an emulsion is almost unlimited. Such an emulsion can be made which contains 99% of oil dispersed in 1% of water.

Various solid materials such as calcium carbonate and basic iron sulfate can be used to stabilize emulsions. Either the water or oil collects on the surface of the dispersed phase forming a membrane which prevents coalescence. Wetting is also important. If a finely divided solid such as lampblack is more easily wetted by oil than by water it should be capable of forming emulsions with water as the disperse phase. Powders have been designated as hydrophilic if they are preferentially wetted by water and stabilize an oil-in-water emulsion. They have been designated as hydrophobic if they stabilize a water-in-oil emulsion.

When interfacial tension between phases is low, emulsions are stable, and any substance which materially lowers the interfacial tension between water and oil will tend to stabilize an emulsion. Thus, lauric acid is the first one in the fatty acid series to greatly lower the surface tension of water when a small concentration of the sodium salt is present and it has been shown to be the first acid of the series to produce an appreciable emulsifying effect. The action of efficient emulsifiers has been ascribed to the collection of the emulsifying agent in the interfacial film. This forms a shell of oriented molecules of the emulsifier around the oil droplets. This is described as the molecular orientation theory of stabilization of emulsions.

The causes of separation of emulsions have been enumerated as: (*1*) adding the oil too rapidly, (*2*) lack of regulation of the conditions of agitation, (*3*) effect of heat during storage, (*4*) effect of freezing during storage, (*5*) agitation during transportation, and (*6*) bacterial spoilage. The stability of salad dressings and mayonnaise has been regulated by the use of an especially designed mobility apparatus.

(*b*) *Homogenization*

Emulsions usually contain dispersed globules which vary greatly in diameter. The process of reducing these globules to a much smaller and approximately equal diameter in an emulsion is termed homogenization. Subdivision of emulsified globules requires a force which increases enormously as the size of the resultant globules decreases. This process has been studied and applied to a greater extent in industrial work than in research work. Laboratory homogenizing equipment is not as satisfactory at present as are industrial homogenizers, or viscolizers as they are sometimes called. A typical technical illustration of the effect of fine subdivision in emulsions is the homogenizing of milk and cream which have long been familiar commercial products. The temperature at which homogenization is conducted influences the size of the resultant particles. A higher temperature produces smaller globules. Homogenization increases the stability of the resultant emulsion. Very little creaming takes place in homogenized milk, while raw milk, of course, creams rapidly. It is even difficult to separate cream from homogenized milk by centrifuging Cream is frequently homogenized, but skim milk cannot be homogenized. Homogenized milk cannot be churned into butter, and homogenized cream cannot be whipped. It is necessary to add a colloid such as gum tragacanth in order to produce a permanent foam on homogenized cream.

The cause of these remarkable changes in properties is the greater adsorption of milk proteins, particularly casein, on the increased number of smaller fat globules in homogenized products. The number of fat globules may be increased more than a thousand times, and the increased adsorbing interface is enormous. The adsorption of casein is manifested by the large increase in viscosity since homogenized milk and cream are much thicker than the original liquids. Homogenized products show no apparent change in density or electrical conductivity. The osmotic pressure may be slightly reduced.

(c) *Antagonism*

Sodium chloride solutions greatly decrease interfacial tension of an oil such as olive oil as shown by stalagmometer measurements with the resultant formation of small droplets. On the other hand, calcium chloride solutions increase the interfacial tension with the accompanying formation of large droplets. These actions may be explained by the formation of small amounts of sodium or calcium soaps. When mixed, sodium and calcium chloride antagonize each other and nullify the effect of both so that there is no resultant effect on interfacial tension. This phenomenon is designated as an antagonism of ions. Calcium antagonizes sodium and magnesium, and potassium antagonizes sodium. The antagonistic action of ions in the precipitation of colloidal sols is similar to their effect on biological phenomena.

(d) *Breaking Emulsions*

Although the stabilization of emulsions is important from an industrial standpoint, the converse problem of breaking emulsions is also important. Emulsions are obtained frequently during industrial processes. No absolutely general rule can be formulated for either making or breaking emulsions. Each such emulsion is usually an individual problem.

Alteration in the orientation of the surface film surrounding the suspended droplets of emulsions and changes in the interfacial tension between the two components are important in the breaking or the inversion of emulsions. A knowledge of surface chemistry and of the role of interfacial tension and a knowledge of the antagonistic effects of ions are useful to the industrial chemist in dealing with these problems.

There are a number of types of methods utilized for the breaking of emulsions including mechanical, chemical, and electrical methods. Centrifuging, which is a common mechanical method, is influenced by such factors as the viscosity of the dispersed phase and the nature and quantity of the emulsifying agent. Preheating is sometimes employed before centrifuging, the decreased viscosity of the dispersed medium being helpful in permitting coalescence of globules. Filtration methods depend on the preferential wetting of a porous or powdered solid material. Porous diaphragms are sometimes used in filtration.

Chemical methods of breaking an emulsion are based on the principle that when an emulsion of water-in-oil is stabilized by a hydrophobic colloid, the addition of a suitable hydrophilic colloid can bring about coalescence. Water-soluble soap is a common agent employed in the chemical treatment of emulsions. Gelatin and starch have also been used. The effectiveness of such methods cannot be definitely predicted but must be determined by trial and error.

Electrolytes are sometimes used to break emulsions. The charge on the globules is neutralized and coalescence results. Common salt and other salts including sodium sulfate and ferric chloride are used to "salt out" the stabilizing film surrounding the emulsion globules.

Electrical methods of breaking emulsions are based on the fact that coalescence of water globules may follow the passage of an electrical current through the emulsion. High potential alternating current is usually employed. The emulsion to be broken is passed continuously through an especially constructed shaft and a potential difference of as high as 11,000 volts is sometimes applied. Distance between the electrodes, voltage, and temperature are factors in the efficiency of this process.

The preparation of butter affords an excellent study of the breaking of an apparently stable emulsion by merely agitating, and the resultant new distribution of emulsifying agents. Churning of cream is intimately related to the formation of foam. Cream first thickens because of the aggregation of the fat. When the fat agglomerates to the necessary dimensions, the emulsion suddenly breaks, and the butter forms. Churning time is 40 to 60 min. The main factors in churning are the fat content, the acidity, the viscosity of the cream, and the vigor of the agitation. Cream containing large fat globules churns the easiest. As the viscosity of cream diminishes, churning becomes easier. The reader is referred to Clayton[38] for an extensive discussion of the theory and application of emulsions.

7. Foams

Froth, or *foam*, is the more or less stable extended air-liquid interface arising when bubbles persist at the surface of a liquid as a result of agitation, aeration, or ebullition. An enormous film surface of liquid is presented to the gas phase so that froth is primarily a labile system owing to the tendency for the interface to be reduced to a minimum. No pure liquid will yield a froth. The chief factors necessary for production of foams are a certain degree of viscosity of the liquid used and a low air-liquid surface tension. High viscosity alone is not sufficient but it must be accompanied by a low surface tension. The froths encountered in food technology are connected with systems in which the surface tension of water is lowered. As a consequence, there is an accumulation of solute at the air-liquid boundary or interface. Frequently the stability is increased by adsorption of the solute which undergoes irreversible changes.

(a) *Egg White Foam*

Perhaps the two most important foams involved in studies of emulsions are beaten egg white and milk froth. The stability when subject to agitation during transportation by road or by rail may be adversely affected. The foaming of egg white or its solution during agitation or whipping is accompanied by an adsorption of protein at the air-water interface. The stability of foams is increased by the addition of acids and acid salts. Egg yolk reduces the foaming of egg white. St. John and Flor[39] showed that the thin or watery part of the white gives a larger volume when beaten than the thick portion of egg white, and also that a larger volume is obtained by whipping at room temperature than when the egg is whipped at icebox temperature.

---

[38] W. Clayton, *Theory of Emulsions and Their Technical Treatment*. 3rd ed., Blakiston, Philadelphia, 1935.

[39] J. L. St. John and I. H. Flor, *Poultry Sci.*, 10, 71 (1930).

(*b*) *Milk Foam*

Fresh milk froths readily. Agitation of milk produces an extended air-liquid interface at which there is an adsorption of a special nitrogenous colloid found in milk. The foam shows considerable permanence because of denaturization of the adsorbed protein. The foaming is affected by temperature. The greater portion of the foam colloid is in the cream because of the large adsorption occurring at the enormous fat-water interface. Churning renders this special colloid inactive. A relation between foam stability and viscosity has been shown. The optimum temperature of frothing lies within the range of 30 to 60° C. The tendency to foam is at a minimum between 20 and 30° C., and there is a decrease below 20° C. The foam on warm milk is different from that on cold milk. It has been shown that the film that forms on heated milk is not entirely due to albumin as has been supposed. Froth on skim milk was found to contain a larger amount of protein. The inorganic constituents were not different in amount. New milk froth is often a technical nuisance. Foam on milk is a disadvantage during pasteurization. Devices are employed by dairies to avoid froth. One method is to run milk into a detention tank drawing the milk off continuously from the bottom. See Volume II, Chapter XX.

(*c*) *Whipped Cream*

Whipped cream is a special case of milk foam. The stability and high viscosity of whipped cream are due to the cell-like structure imparted to the bubbles and these are stiffened by the denaturization of the adsorbed protein and also by a quantity of partially solidified milk fat. The whipping capacity of cream is improved by aging of the cream, increase in fat content, and increase in solids-not-fat. Aging of cream assists clustering. Factors which increase the viscosity of cream also improve the whipping capacity. Homogenization of cream reduces the whipping capacity. Although surface tension of milk and cream cannot always be definitely related to whipping properties, a decreased surface tension is usually associated with improved whipping capacity. The reduction of whipping capacity by pasteurization is related to its effect in breaking up clusters of fat globules The duration of whipping influences the volume of whipped cream which rises rapidly to a maximum in about 3 mins. The maximum stability occurs after a longer period of whipping.

8. Other Edible Emulsions

The food industry provides many instances of the use of emulsions. Milk is the best-known natural emulsion and offers a very interesting field for investigation, partly because of the variety of questions it raises in colloidal physics and chemistry. Milk is not only a colloidal sol containing emulsified fat, but also contains both protein and carbohydrates in solution or as colloidal sols. In ice cream manufacture, milk protein acts as an emulsifying agent for the fat and produces smooth texture. If gelatin is added, a harder ice cream results with a higher melting point. The hardest and most heat-resisting ice cream is given by a medium per cent of fat and a large amount of gelatin. An increase in the gelatin concentration in ice cream increases the viscosity, but not in a corresponding ratio. Egg albumen may be added as a protective colloid. Egg yolk improves the whipping qualities of the milk and facilitates freezing.

The chief edible emulsions are milk and cream, salad dressing and mayonnaise, and certain medicinal emulsions. Emulsions of essential oils for flavoring purposes have been prepared. Emulsions of cod-liver oil were famous in years past, and were used for human and animal feeding. Shortening agents have been prepared in emulsified form. Lecithin has been used as an emulsifying agent in foods. In food emulsions, the enormously increased oil-water interface present introduces problems of rancidity. It has been calculated that the fat-water interface in 1 lb. of butter may equal 1000 square meters. The rancidity of butter has been connected with the extent of the aqueous phase. Margarine is an emulsion of vegetable oils used instead of butter. Although this is an oil-in-water emulsion, a recent tendency is to emulsify the correct amount of water directly in the melted oil phase using a suitable emulsifying agent.

Salad dressings may be either thin flowing (salad creams) or viscous (mayonnaise). Salad creams may be made from a variety of ingredients. Mayonnaise is generally understood to be made from eggs, oil, vinegar, and flavoring agents. In the finished product, the sum of the percentages of vegetable oil and fresh egg yolk, free from white, is not less than 50%. The quantity of any stabilizing material used does not exceed 2%. The published analyses of salad dressings and mayonnaise reveal wide variety in amounts of the various ingredients. These products are also of the oil-in-water type of emulsion. Egg is the principal emulsifying agent. Egg white has only one-fourth the emulsifying capacity of the yolk. A special egg-yolk preparation for salad creams contains glycerol and acetic acid. The importance of mustard as an efficient solid powder emulsifier has been demonstrated.

IV. FOODS AS SOLUTIONS AND MIXTURES

Most foods are consumed in the solid form, although some are consumed as liquids, such as milk, fruit juices, and other beverages. Of these latter, some, although comparatively few, are true chemical solutions. Many are mixtures of solutions and sols or suspended solids. Natural fruit juices, such as grape juice and apple cider, are primarily chemical solutions, although modern commercial fruit juices according to the new conception pointed out by Neubert and St. John (Vol. II, Chapter XIX) contain large quantities of suspended fruit solids. The liquid portion of canned fruits is also largely a solution of sugars. Natural foods such as honey and maple sirup are supersaturated solutions of several different sugars; also much of the dispersed solid material may be in colloidal condition. The same may also be true with manufactured products such as molasses and corn sirup. Beverages such as coffee, tea, and cocoa are marketed in solid form but are converted by the consumer to solutions which probably also contain some material in colloidal and suspended form. The manufacturer must control these products so that he can be sure that the consumer will be able to prepare a suitable product for consumption. Many soft drinks are solutions or contain a large portion of their components in solution. Commercial canned soups also contain a part of their ingredients in solution, although some contain much colloidal, suspended, and solid ingredients. Strained baby foods may contain some material in solution but are primarily suspensions or emulsions. Certain foods like eggs contain some ingredients in solution, but are largely gels or sols. Foods such as lean meat retain an appreciable amount of body fluids in which are dissolved inorganic and some organic constituents, and they are therefore partly solutions, although largely in the gel form. A few commercial products are utilized by the consumer in liquid form, such as vegetable oils which are mixtures of fats. Of the principal classes of food materials, two—carbohydrates and fats—are in various cases consumed primarily as such. Most natural foods, however, are mixtures of carbohydrates, proteins, fats, inorganic components, vitamins, and water. The chemical composition of several of the vitamins is now known. Foods have one or more of these components in the solid form, or in solution, or in the form of a colloidal sol or emulsion.

Selected Bibliography

Alsberg, C. L , "Physical Chemistry as Applied to Foods," *Ind Eng Chem* , **14,** 867 (1922)

Bailey, C. H , *The Chemistry of Wheat and Flour.* Chemical Catalog Co , New York, 1925

Bingham, Eugene C., *Fluidity and Plasticity.* McGraw-Hill, New York, 1922

Browne, Charles A , and Zerban, F. W., *Physical and Chemical Methods of Sugar Analysis* Wiley, New York, 1941.

Clark, W. M., *The Determination of Hydrogen Ions.* 3rd ed., Williams & Wilkins, Baltimore, 1928

Clayton, W., *Colloid Aspects of Food Chemistry and Technology.* Van Nostrand, New York, 1937

Clayton, W , "The Concept of 'Colloid' and Its Importance for the Food Chemist," *Food Manuf* , **4,** 219, 251, 350 (1929), **5,** 79, 160 (1930)

Clayton, W., *Theory of Emulsions and Their Technical Treatment.* 3rd ed , Blakiston, Philadelphia, 1935.

Cruess, W. V., *Commercial Fruit and Vegetable Products.* 2nd ed , McGraw-Hill, New York, 1938

Gortner, R. A , *Outlines of Biochemistry.* Wiley, New York, 1938.

Jacobs, M. B., *Chemical Analysis of Food and Food Products.* Van Nostrand, New York, 1938.

Kolthoff, I. M., and Laitinen, H. H., *pH and Electro Titrations; The Colorimetric and Potentiometric Determination of pH.* 2nd enl. and rev. ed., Wiley, New York, 1941.

Leach, A. E., *Food Inspection and Analysis.* Wiley, New York, 1920.

Lewkowitsch, M. A., *Chemical Technology and Analysis of Oils, Fats, and Waxes.* Macmillan, London, 1921.

McCollum, E. V., Chap. 29 in Robert H. Bogue, *Colloidal Behavior.* Vol. 2, McGraw-Hill, New York, 1924.

Michaelis, L., *Hydrogen Ion Concentration.* Williams & Wilkins, Baltimore, 1936.

Morrow, C. A., and Sandstrom, W. M., *Biochemical Laboratory Methods for Students of the Biological Sciences.* Wiley, New York, 1935

Prescott, S. C., and Proctor, B. E., *Food Technology* McGraw-Hill, New York, 1937

Willows, R. S., and Hatschek, E , *Surface Tension and Surface Energy.* 3rd ed , Churchill, London, 1923.

Chapter III

THE CARBOHYDRATES

ED. F. DEGERING

Lafayette, Indiana

I. DEFINITION AND FORMULATION

The simplest member of the carbohydrate series is glycolaldehyde ($HO \cdot CH_2 \cdot CHO$),[1] which is the hydroxy derivative of ethanal and known, consequently, as hydroxyacetaldehyde or hydroxyethanal. The next members of the series are glyceraldehyde ($HO \cdot CH_2 \cdot CHOH \cdot CHO$) and dihydroxyacetone ($HO \cdot CH_2 \cdot CO \cdot CH_2 \cdot OH$). The former is known also as 2,3-dihydroxypropanal and the latter as 1,3-dihydroxypropanone.

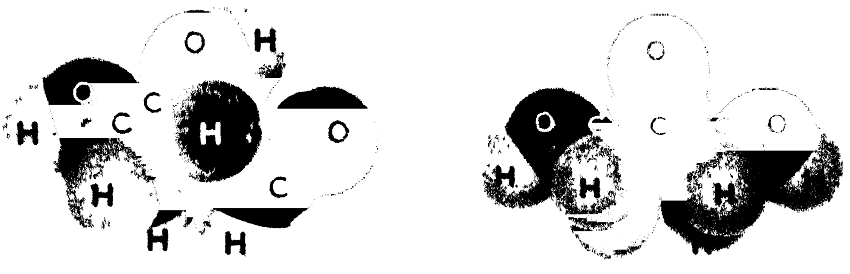


Fig. 9. Fischer-Hirschfelder atomic models of simple sugars.
2,3-Dihydroxypropanal 1,3-Dihydroxypropanone

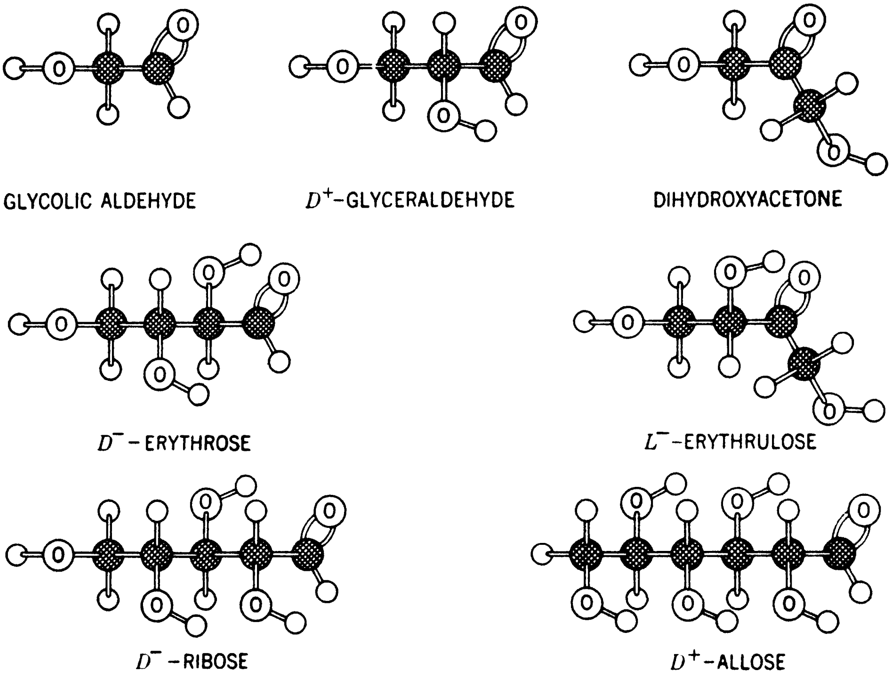
To the simplest member, glycolaldehyde, is applied the term *diose* (biose) because it contains two carbon atoms per unit structure.[1] Glyceraldehyde and dihydroxyacetone accordingly are trioses, but the former is an aldotriose whereas the latter is a ketotriose. Ascending the series are the aldotetroses as $HO \cdot CH_2 \cdot CHOH \cdot CHOH \cdot CHO$, the aldopentoses as $HO \cdot CH_2 \cdot CHOH \cdot CHOH \cdot CHOH \cdot CHO$, and the aldohexoses as $HO \cdot CH_2 \cdot CHOH \cdot CHOH \cdot CHOH \cdot CHOH \cdot CHO$. Formulas for ketotetroses, ketopentoses, and ketohexoses may be written, but with the exception of *D*-

---

[1] A detailed consideration of nomenclature is given on page 64.

fructose ($HO \cdot CH_2 \cdot HCOH \cdot HCOH \cdot HOCH \cdot CO \cdot CH_2OH$) they are comparatively unimportant.

From these specific examples of carbohydrate material one might infer that this series may be represented by the general formula $C_n(H_2O)_n$, and that the carbohydrates are polyfunctional compounds containing one carbonyl group with each of the other carbon atoms supporting a hydroxyl group.


GLYCOLIC ALDEHYDE

D⁺-GLYCERALDEHYDE

DIHYDROXYACETONE

D⁻-ERYTHROSE

L⁻-ERYTHRULOSE

D⁻-RIBOSE

D⁺-ALLOSE

Fig. 10. Tinker-toy representations of the first few members of the monosaccharide series.

The first observation, which is basically true only for the monosaccharides, led to the adoption of the term carbohydrate, which implies *hydrates of carbon*. That the name is a misnomer is evidenced by the facts that (*1*) there are carbohydrates that do not conform to the hydrate rule, (*2*) there are no true hydrates among the carbohydrate series,[2] and (*3*) there are compounds that conform to the hydrate rule that are not true carbohydrates. Exceptions of the first type are afforded by rhamnose [$C_6H_2(H_2O)_5$] and rhamnoheptose [$C_7H_2(H_2O)_6$]. In confirmation of exception two, no hydrate systems of the type $C_n(H_2O)_n \rightleftarrows n\,C + n\,H_2O$ are known among the members of the carbohydrate family, whereas analogous systems are common among the true hydrates as illustrated by $Na_2SO_4 \cdot 10H_2O \rightleftarrows Na_2SO_4 + 10H_2O$. Exceptions of the third type are illustrated by the formulas for formaldehyde ($H \cdot CHO$), acetic acid ($CH_3 \cdot CO_2H$), and lactic acid ($CH_3 \cdot CHOH \cdot CO_2H$).

---

[2] This should not be confused with the hydrates formed by the addition of one or more equivalents of water to a sugar molecule.

The carbohydrates may be represented, in general, by the type formula $n[C_x(H_2O)_x] - (n - 1)H_2O$, where x is the number of carbon atoms in the building unit and n is the number of building units per molecule. For the hexoses x has a value of 6 and for the disaccharides n has a value of 2.

The polyfunctional nature of these compounds affords the basis for their definition. The carbohydrates are, actually or potentially, hydroxy or polyhydroxy oxo derivatives of the hydrocarbons. The simpler members (as the monosaccharides) are *actually* hydroxy or polyhydroxy aldehydes or ketones, whereas the more complex members (as the di-, tri-, and polysaccharides) are *potentially* polyhydroxy aldehydes and/or ketones because such products result from hydrolysis.

II. OCCURRENCE AND USES

Glycolaldehyde may occur as a metabolic intermediate from hydrolytic deamination and biological reduction of glycine ($H_2N \cdot CH_2 \cdot CO_2H$), and D^+-glyceraldehyde is doubtless formed in the biochemical utilization of the simple sugars. L^+-Arabinose and D^+-xylose are widely distributed in the form of their intermolecular anhydrides, the arabinosans and the xylosans. The former may be obtained from cherry gum and gum arabic, and the latter from bran, corncobs, cottonseed hulls, oat hulls, peanut shells, straw, and most woods. D^+-Glucose occurs as such in ripe fruits, flowers, honey, leaves, roots, sap, urine, and blood (0.1%), and in the form of its intermolecular anhydrides, as dextrins, glycogen, starch, and cellulose. D^+-Galactose occurs in agar-agar, in the cerebrosides of the brain, and as one of the building units of lactose and raffinose. D -Fructose is the building unit of inulin, is found in honey, and is one of the building units of sucrose and raffinose. Sucrose occurs in almonds, coffee, flowers, honey, maple sap, sugar beet, sorghum cane, sugar cane, walnuts, and in most plants. Maltose is found in beer, in corn sirup, in malt, and is one of the hydrolytic products of starch. Lactose occurs in milk (3 to 7%). Dextrins occur in honey, in vegetable juices, in some plants, and among the hydrolytic products of starch. Glycogen is found in the liver (about 10%), in muscle tissue, and in small amounts in the blood, brain, kidneys, pancreas, spleen, and also in fungi. Starch is present in practically all plants, particularly in the storage cells as the seeds and tubers. Cellulose is the structural material of the cell walls of plants. Inulin occurs in certain lichens, chicory root, dahlia bulbs, dandelion roots, and Jerusalem artichokes.

The carbohydrates are one of the most important sources of food and raw materials as evidenced by the multiplicity of their uses. D^+-Glucose, D^--fructose, D^+-galactose, maltose, lactose, sucrose, glycogen, dextrin, and starch are the carbohydrate materials that are utilized by the higher forms of life as a source of heat and energy. The pentoses are digested by some of the herbivorous animals and are the raw materials for the commercial production of thousands of pounds of furfural per year. The 70–80% commercial glucose is used in the silvering of mirrors, as a crystallization inhibitor for sodium sulfate in the rayon precipitating bath, and in the fermentative production of ethyl alcohol, acetic acid, lactic acid, citric acid, and other products.

Lactose is present in or is used to preserve many foods. It is a coating agent for olives, preserved fruits, and sugared almonds, a flavoring agent in chocolate products, and a firming agent for soft fruits. It is an ingredient of baking and biscuit mixes, of some cheese, of dry coloring material for edible fats, of infant and invalid foods, and of some soups. It is a preservative for flavor, color, and consistency in meat products, and is a substitute for other sugars in jam manufacture. Other than as a food, lactose is used in bath salts, in certain dentifrices, in ensilage, in explosives, in matches, in making mirrors, and as a preservative for latex. In the soap industry it is used as a stabilizer for color, emulsification, and transparency.

Maltose is used as a food in beer and malt production, in making beverages and soft drinks, in bread doughs, in confections, as a dehydrating agent for fruit juices, in infant and invalid foods, in jams, in milk and coffee substitutes, in tea extracts, and in yeast. It is an important process material in making chemicals, fungicides, and insecticides. Maltose is an ingredient of bee food, dyes, fertilizers, leather goods, pharmaceutical materials, printing compositions, snuff, and stamp-pad compositions. In the textile industry it is used in textile dyeing and printing.

The importance of sucrose is best illustrated by its historical development. The use of honey, milk, and fruits doubtless represents the beginning of sugar consumption by the human race. Sugar cane was known in ancient China, India, and Egypt. As early as the 7th century sugar had already become a commercial product. In the 16th century the culture of sugar cane was introduced into Brazil and the West Indies. At present it is produced in Australia, Brazil, China, Cuba, Egypt, India, Jamaica, Japan, Peru, the Philippine Islands, other tropical countries, and some of the southern States. Most of the sucrose is used in the confectionery trade, in the food industries, and as a sweetening agent for all types of foods and internal medicinals. Sucrose octaäcetate is used as a plasticizer and as a denaturant. Other derivatives find a limited use.

Dextrin is used in dye pastes as a thickener, in many kinds of inks, and in the sizing of paper, cardboard, felt, and numerous textiles. It is also used in textile printing. It finds use alone as an adhesive and in many glues and pastes. It is an ingredient of bricks, porcelain, leather finishes, tanning extracts, and shoe and metal polishes. Dextrin is used as an absorbent in explosives, and in matchhead and pyrotechnic compositions. Dextrin is an ingredient in bakery products and confections. It is used in polishing barley, coffee, and rice. It is an ingredient of silvering compounds for glass. It may be used as a substitute for gum arabic, gum tragacanth, and other gums. It is a starting point in making emulsifying agents.

Starch has many uses, the more important of which are in laundering, as an ingredient in artificial leather, cattle foods, chemical preparations, coatings for rubber, compositions for removing boiler scale, dental impression material, some explosives, many foods, glues, insecticides, leather cleaning compositions, pastes, sachets, sizings, silk dyes, soaps, and toilet powders. It is also used in the manufacturing of dry-cell batteries. Starch is the starting point in making fermented and distilled liquors, in the commercial production of butanol and butanediols, in making certain analytical reagents, nitrostarch explosives, caramel, malt sugar, various sirups and mixtures. It is used as a binder in the making of fuel briquets, and as an ingredient in certain road surfacing materials. In the textile industry, starch is used in the sizing of thread, in dyeing, in printing, and in fireproofing. Starch from potatoes finds special uses in flour-bleaching mixtures, in nitrostarch lacquer compositions, and as a dispersing agent in making waxed paper. It is also used in bituminous emulsions. Cornstarch has a special use as a starting material for making driers for paint and varnish.

Cellulose[3] is an ingredient of coating compositions used for protecting and decorating ceramic products, fibrous products, metalware, paper and paper products, rubber goods, stonework, textiles, wood products, and woodwork. It is also an important insulating material. It is used in making adhesives, building materials, bronzing liquids, cements, dopes, enamels, glues, lacquers,[4] lumber, mercerized cotton, parchment paper, plastics, shatterproof glass, varnishes, and vulcanized rubber.

Cellulose acetate finds special uses in the manufacture of artificial pearls, Celanese rayon, electrical condensers, filaments, noninflammable films, noninflammable plastics, phonograph records, and various novelties. It is used as a coating for glass (to prevent fogging) and in fireproof fabrics. Cellulose palmitate and cellulose stearate are both used as thickeners for cup greases and heavy-duty lubricating oils. The foremost uses of nitrocellulose are: in celluloid and other plastics, in explosives, in nail enamels and lacquers, in sheet and roll films, in the manufacture of solidified alcohols for fuel, and as an intermediate for various forms of rayon. Nitrocellulose is used as the starting material in making collodion and soluble pyroxylins.

III. STRUCTURE OF MONOSACCHARIDES

1. Principles and History of Structure Determination

The structure of a monosaccharide may be established systematically by determining in order the empirical, the molecular, the skeletal, the semiconstitutional, the constitutional, the semistructural, and the structural formulas by proper interpretation of adequate experimental data.

A quantitative analysis of purified D^+-glucose, for example, gives 40.0% carbon, 6.7% hydrogen, and 53.3% oxygen, from which the empirical formula is calculated to be CH_2O. It was this formula that suggested the French term *hydrate de carbone* which became *Kohlenhydrat* in German and *carbohydrate* in English.

With the appearance of the Beckmann apparatus in 1888, Tollens and Meyer succeeded in applying the lowering of the freezing point of a solution to the determination of the molecular weight of D^+-glucose. Their data indicated a value of about 180 which suggests six empirical units or $(CH_2O)_6$ or $C_6H_{12}O_6$ as the molecular formula.

---

[3] For special uses of cellulose, dextrin, lactose, maltose, and starch, see T. C Gregory, *Uses and Applications of Chemical and Related Materials*. Reinhold, New York, 1939. According to C. M. A Stine of du Pont de Nemours and Co, "Ten thousand individual articles are made from cellulose and the industry is still in its infancy"

[4] The use of Duco has reduced the time required to paint an automobile from 20 days with varnish to a few hours with lacquers.

Two years before the molecular weight determination of D^+-glucose by Tollens and Meyer, Kiliani had succeeded in improving the cyanohydrin synthesis of Schutzenberger to the extent that he obtained the cyanohydrin of D^+-glucose, which was converted to the corresponding acid by hydrolysis. The acid was then reduced (1886), with concentrated hydrogen iodide in the presence of red phosphorus, to yield *n*-heptanoic acid, the equations being:

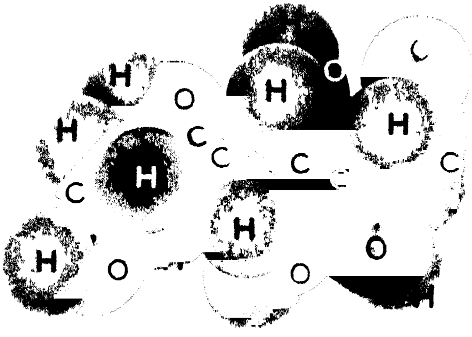


Fig 11.—D^+-glucose as represented by a Fischer-Hirschfelder model

$$D^+\text{-glucose} + H\cdot CN/(\text{trace of } NH_3, \text{aq}) \longrightarrow D^+\text{-glucose cyanohydrin, then}$$
$$D^+\text{-glucose cyanohydrin} + 2H_2O/H^+, \text{aq.} \longrightarrow D^+\text{-glucoheptanoic acid} + NH_4^+, \text{and}$$
$$D^+\text{-glucoheptanoic acid} + 12HI/4P, \text{heat} \longrightarrow CH_3\ CH_2\ CH_2\cdot CH_2\ CH_2\ CH_2\cdot CO_2H + 4PI_3 + 6H_2O$$

From these data it is apparent that the skeleton formula of D^+-glucose is correctly represented by C·C·C·C·C·C; for *n*-heptanoic acid $(CH_3\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH_2\cdot CO_2H)$ is known to contain a continuous carbon chain.

In 1879 Franchimont succeeded in obtaining a crystalline pentaacetate derivative, $C_6H_7O(OAc)_5$, of D^+-glucose. Such a derivative suggests the presence of five alcoholic hydroxyl groups in the glucose molecule,[5] hence the semiconstitutional formula for D^+-glucose may be written as $C_6H_7O(OH)_5$. This acetylation reaction is frequently employed in analytical chemistry to determine the number of alcoholic hydroxyl groups, the compound showing a net increase in molecular weight of 42 for each acetyl group introduced.

---

[5] Acylation is used for the quantitative estimation of the alcoholic hydroxyl group, the equation being: $R\cdot OH + Ac_2O \text{ (or } Ac\cdot Cl) \rightarrow R\cdot O\cdot Ac + Ac\cdot OH \text{ (or } HCl)$

The acetyl chloride is more severe than the anhydride because the by-product hydrochloric acid catalyzes hydrolysis of the complex molecules.

By the same series of reactions by which Kiliani proved the skeleton formula for D^+-glucose (1886), he showed that it is an aldohexose; for only an aldohexose can yield a heptanoic acid derivative, as indicated by:

$$\begin{aligned}
&\mathrm{HO\cdot CH_2\cdot(CHOH)_4\cdot CHO + H\cdot CN/(trace\ NH_3)} \longrightarrow \mathrm{HO\cdot CH_2\cdot(CHOH)_4\cdot CHOH\cdot CN}, \text{ then}\\
&\mathrm{HO\cdot CH_2\cdot(CHOH)_5\cdot CN + 2H_2O/(H^+, aq.)} \longrightarrow \mathrm{HO\cdot CH_2\cdot(CHOH)_5CO_2{}^{-}{}^{+}NH_4}, \text{ then}\\
&\mathrm{HO\cdot CH_2\cdot(CHOH)_5\cdot CO_2H + 12HI/4P}, \text{ heat} \longrightarrow \mathrm{CH_3\cdot(CH_2)_5\cdot CO_2H + 6H_2O + 4PI_3}, \text{ but}\\
&\mathrm{HO\cdot CH_2\cdot CHOH\cdot CHOH\cdot CHOH\cdot CO\cdot CH_2\cdot OH + HCN + 2H_2O/(H^+) + 12HI/4P}, \text{ heat} \longrightarrow\\
&\qquad\qquad \mathrm{CH_3\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH(CH_3)\cdot CO_2{}^{-} + 6H_2O + 4PI_3 + NH_4{}^{+}}
\end{aligned}$$

The constitutional formula of D^+-glucose,[6] then, may be written as

$$\mathrm{HO\cdot CH_2\cdot(CHOH)_4\cdot CHO} \text{ or } \mathrm{HO\cdot CH_2\cdot CHOH\cdot CHOH\cdot CHOH\cdot CHOH\cdot CHO}$$

Closely related to this experimental work on the structure of D^+-glucose is the theoretical work of van't Hoff and Le Bel (1874). They showed that a compound with different terminal groups which also contains asymmetric groupings will have 2^n isomers, where n is the number of asymmetric groupings. Since the constitutional formula of D^+-glucose indicates the presence of four asymmetric groupings, it is apparent that there should be sixteen isomeric forms. With this historical setting, "the time was ripe for a genius to arise who would possess the ability and the industry to bring order out of chaos, and this genius was Emil Fischer." [7]

While the aforementioned research was in progress, Emil Fischer was busily engaged in studying the chemical properties of phenylhydrazine ($\mathrm{H_2N\cdot NH\cdot C_6H_5}$), a material which he had just synthesized and for which he was seeking new uses (1884–1887). He observed that his new compound gave satisfactory derivatives of aldehydes and ketones, which results suggested its use as a sugar reagent. In 1884 he observed that the reaction of phenylhydrazine on D^+-glucose and D^--fructose gave the same derivative, a glucophenylosazone. This was enough to arouse the interest of a genius, and Fischer assigned himself to the task of elucidating the structure of the sugars. In this he made use of the available data and established new relationships through (*1*) a study of the osazones of the sugars (see page 82), (*2*) reduction of some of the sugars to the *meso* alcohols (see page 79), (*3*) oxidation of some of the sugars to the *meso* acids (see page 80), (*4*) the Kiliani synthesis (see page 76), and (*5*) the Wohl degradation (see page 77).

2. Structure of Aldohexoses

(*a*) *The D^+-Galactose Series*[8]

1. D^+- or L^--Galactose + $\mathrm{HNO_3}$ (sp. gr. 1.15)[9] $\longrightarrow$ an inactive diacid (mucic acid).

---

[6] Both $\cdot\mathrm{CH_2OH}$ and $\cdot\mathrm{CHO}$ are univalent groups and must occupy terminal positions.

[7] H. Gilman, *Organic Chemistry, An Advanced Treatise.* Vol. II, Wiley, New York, 1938, p. 1403.

[8] In the galactose series, the OH groups on carbon atoms 3 and 4 lie on the same side of the molecule.

[9] Nitric acid, sp gr 1.15, oxidizes both terminal groups of a simple sugar to give a hydroxy or polyhydroxy diacid (see page 81).

2. D^+- or L^--Galactose + HCN + hyd. + HNO_3 (sp. gr. 1.15) $\longrightarrow$ two active diacids.

3. D^+- and L^--Galactose + epimeric change[10] $\longrightarrow$ D^+ and L^--talose, respectively.

4. D^+- or L^--allose + HNO_3 (sp. gr. 1 15) $\longrightarrow$ an inactive diacid (allomucic acid).

5. D^+- and L^--allose + epimeric change $\longrightarrow$ D^+- and L^--altrose, respectively.

(*b*) *The D^+-Glucose Series*

1. D^+- and L^--Glucose + inversion[11, 12] $\longrightarrow$ L^+ and D^--gulose, respectively.

2. D^+- and L^--Glucose + H_2NOH + $Ac_2O/AcONa$ + $NH_3/AgNO_3/EtOH$ + HCl, aq $\longrightarrow$ D^-- and L^+-arabinose
   D^-- and L^+-Gulose + H_2NOH + $Ac_2O/AcONa$ + $NH_3/AgNO_3/EtOH$ + HCl, aq. $\longrightarrow$ D^+- and L^--xylose.

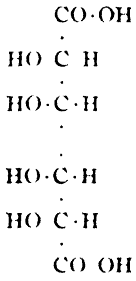
3. D^-- and L^+-Arabinose + HNO_3 (sp. gr. 1 15) $\longrightarrow$ an active diacid (*D*-trihydroxyglutaric acid).
   D^+- and L^--Xylose + HNO_3 (sp. gr. 1.15) $\longrightarrow$ an inactive diacid (*i*-trihydroxyglutaric acid).

4. D^+- and L^--Glucose + epimeric change $\longrightarrow$ D^+- and L^--mannose, respectively.

5. D^-- and L^+-Gulose + epimeric change $\longrightarrow$ D^-- and L^+-idose, respectively

(*c*) *Identification of Possible Configurations*

With these data available and the use of a modified Rosanoff classification chart for sugar structures (see page 69), it is possible to identify one by one the sixteen possible configurations of the aldohexoses required by the van't Hoff-Le Bel postulate.[13]


$$\begin{array}{c} CO{\cdot}OH \\ HO{\cdot}C{\cdot}H \\ HO{\cdot}C{\cdot}H \\ HO{\cdot}C{\cdot}H \\ HO{\cdot}C{\cdot}H \\ CO{\cdot}OH \end{array}$$

By the use of fact *a*.1, the galactose pair must be represented by formulas 1,16 or 7,10 as none of the other configurations will yield the required inactive diacids. If oxidation to give the like terminal groups yields a symmetrical molecule, optical inactivity results, for the molecule is symmetrical with respect to a line or plane. The upper half of the molecule is the mirror image of the lower half, as shown at the right.

From fact *a*.2 it is seen that the galactoses, by the addition of hydrogen cyanide, subsequent hydrolysis, and oxidation, yield two active diacids, as shown by the equations:

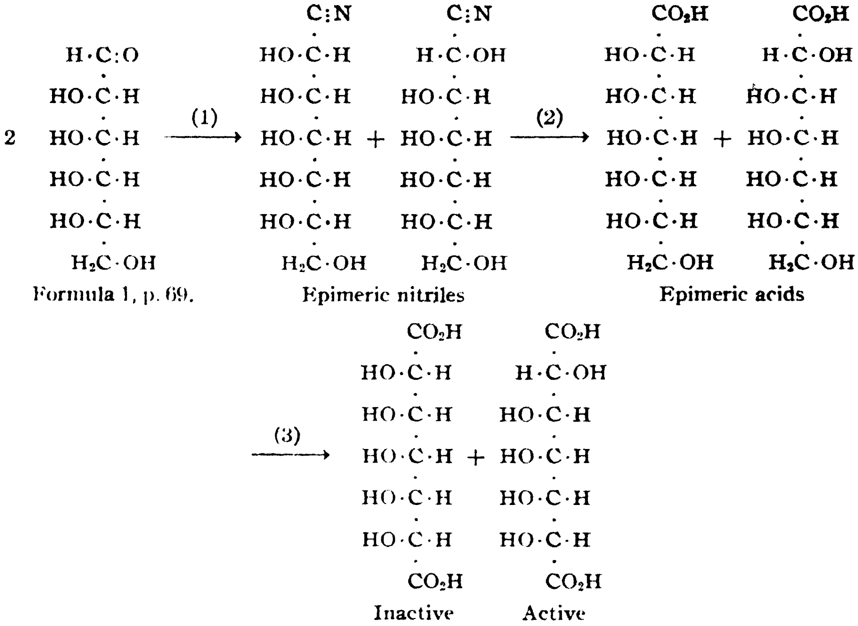
---

[10] See Epimeric change, page 66.

[11] Inversion involves two changes: $\cdot CHO \rightarrow \cdot CH_2OH$, and $\cdot CH_2OH \rightarrow \cdot CHO$. See page 66.

[12] See page 61.

[13] Van't Hoff-Le Bel predicted $2n$ isomers, where n is the number of asymmetric carbon groupings provided the terminal groups are different.



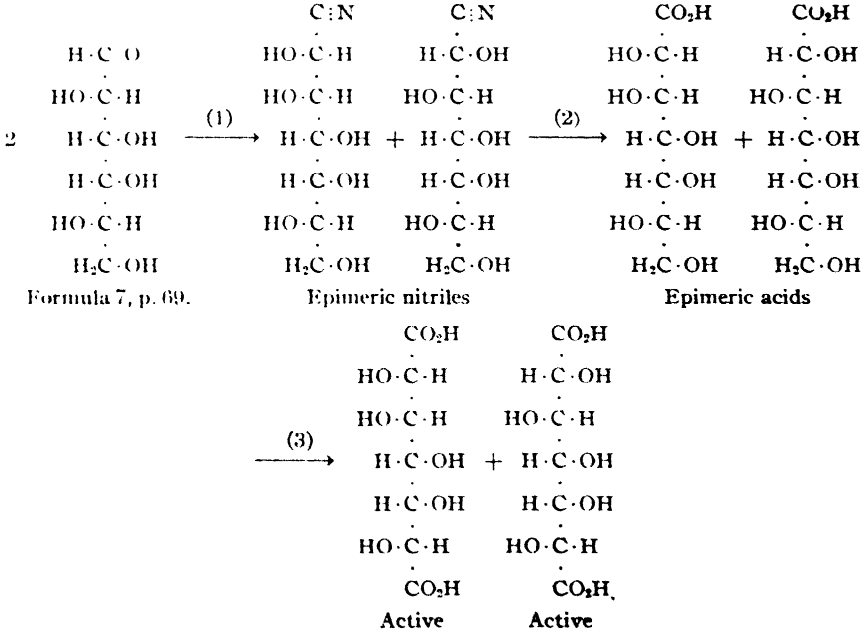
$$
2\ \begin{array}{c} H\cdot C{:}O \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ H_2C\cdot OH \end{array}
\xrightarrow{(1)}
\begin{array}{c} C{:}N \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ H_2C\cdot OH \end{array}
+
\begin{array}{c} C{:}N \\ H\cdot C\cdot OH \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ H_2C\cdot OH \end{array}
\xrightarrow{(2)}
\begin{array}{c} CO_2H \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ H_2C\cdot OH \end{array}
+
\begin{array}{c} CO_2H \\ H\cdot C\cdot OH \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ H_2C\cdot OH \end{array}
$$

Formula 1, p. 69. Epimeric nitriles Epimeric acids

$$
\xrightarrow{(3)}
\begin{array}{c} CO_2H \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ CO_2H \end{array}
+
\begin{array}{c} CO_2H \\ H\cdot C\cdot OH \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ CO_2H \end{array}
$$

Inactive Active

The reagents for the successive steps are: (1) HCN/trace of NH_3, (2) $4H_2O/(H^+)$, and (3) HNO_3 (sp. gr. 1.15).



$$
2\ \begin{array}{c} H\cdot C{:}O \\ HO\cdot C\cdot H \\ H\cdot C\cdot OH \\ H\cdot C\cdot OH \\ HO\cdot C\cdot H \\ H_2C\cdot OH \end{array}
\xrightarrow{(1)}
\begin{array}{c} C{:}N \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ H\cdot C\cdot OH \\ H\cdot C\cdot OH \\ HO\cdot C\cdot H \\ H_2C\cdot OH \end{array}
+
\begin{array}{c} C{:}N \\ H\cdot C\cdot OH \\ HO\cdot C\cdot H \\ H\cdot C\cdot OH \\ H\cdot C\cdot OH \\ HO\cdot C\cdot H \\ H_2C\cdot OH \end{array}
\xrightarrow{(2)}
\begin{array}{c} CO_2H \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ H\cdot C\cdot OH \\ H\cdot C\cdot OH \\ HO\cdot C\cdot H \\ H_2C\cdot OH \end{array}
+
\begin{array}{c} CO_2H \\ H\cdot C\cdot OH \\ HO\cdot C\cdot H \\ H\cdot C\cdot OH \\ H\cdot C\cdot OH \\ HO\cdot C\cdot H \\ H_2C\cdot OH \end{array}
$$

Formula 7, p. 69. Epimeric nitriles Epimeric acids

$$
\xrightarrow{(3)}
\begin{array}{c} CO_2H \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ H\cdot C\cdot OH \\ H\cdot C\cdot OH \\ HO\cdot C\cdot H \\ CO_2H \end{array}
+
\begin{array}{c} CO_2H \\ H\cdot C\cdot OH \\ HO\cdot C\cdot H \\ H\cdot C\cdot OH \\ H\cdot C\cdot OH \\ HO\cdot C\cdot H \\ CO_2H. \end{array}
$$

Active Active

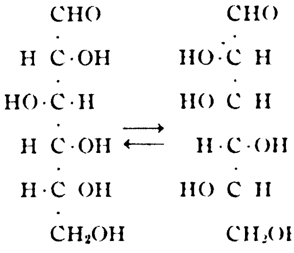
This eliminates the 1,16 pair and leaves only the 7,10 pair as representative of the configuration of the galactoses, formula 7 representing L^--galactose and formula 10 representing D^+-galactose.

Fact *a*.3 indicates that the taloses are related to the galactoses by epimeric change, which involves a transposition of the *H* and the *OH* on the α-carbon grouping (that is, H·C·OH to HO·C·H). If such a change is effected on formula 7, formula 8 is obtained, which represents the configuration of L^--talose. By a similar change, formula 10 gives formula 9, which is the configuration assigned to D^+-talose.

The data represented in fact *a*.4 recall the observations made in connection with fact *a*.1 in which it was found that pairs 1,16 and 7,10 do give inactive diacids. As the 7,10 pair has now been assigned to the galactoses, the 1,16 pair must represent the L^-- and D^+-form of the alloses.

From fact *a*.5 it is apparent that formula 2 must be assigned to L^--altrose and formula 15 to D^+-altrose. This leaves only formulas 3, 4, 5, 6, 11, 12, 13, and 14 for further consideration.

From fact *b*.1 it is observed that D^+-glucose is related to L^+-gulose by *inversion*.[14] Inversion is defined as the equivalent of the rotation of the asymmetric nucleus in the plane of the paper. Actually, the aldehyde group is reduced to a primary alcohol group, and the primary alcohol group initially present is oxidized to an aldehyde group (·CHO → ·CH_2OH and ·CH_2OH → ·CHO). By inspection it is seen that inversion of formula 3 yields formula 12, formula 5 gives formula 14, and both formulas 4 and 6 yield themselves. If, then, formula 3 represents L^--glucose, formula 5 must be assigned to L^+-gulose, and if formula 5 is the configuration of L^--glucose, then L^+-gulose must be represented by formula 3.


Net result of inversion

It is seen from fact *b*.2 that L^--glucose is related to L^+-arabinose and L^+-gulose is related to L^--xylose. If, then, either of these pentoses can be identified, the hexoses may be identified by their synthetic relationship.

Fact *b*.3 reveals that the arabinoses yield active diacids whereas the xyloses give inactive diacids. It is apparent, therefore, that formula *B* must be assigned to L^+-arabinose and that formula *C* must be assigned to L -xylose. But if formula *B* represents the structure of L^+-arabinose, then formula 3 must be assigned to L^--glucose, and if formula *C* indicates the configuration of L^--xylose, then formula 5 must be assigned to L^+-gulose. The mirror image of 3 is 14, which represents D^+-glucose, and the mirror image of 5 is 12, which represents D^--gulose.

According to fact *b*.4, the glucoses and mannoses are related by epimeric change. Since formula 3 represents L^--glucose, its epimer L -mannose must be represented by formula 4. D^+-Mannose, consequently, is represented by the configuration of formula 13.

---

[14] See Inversion, page 66.

By fact *b*.5 the epimers of the guloses are the idoses; hence formula 6 represents the configuration of L^+-idose and formula 11 that of D^--idose. By this line of reasoning, the sixteen possible configurations of the aldohexoses have been assigned to the respective sugars.

Of these sixteen isomeric sugars, eleven of them were isolated or synthesized and identified by Emil Fischer and his students. Inasmuch as these eleven included either a *D*- or *L*-form of the eight distinct sugars, it was possible to assign structures to all of the sixteen possible isomers. The synthesis of the last aldohexose and its identification was reported in 1935.

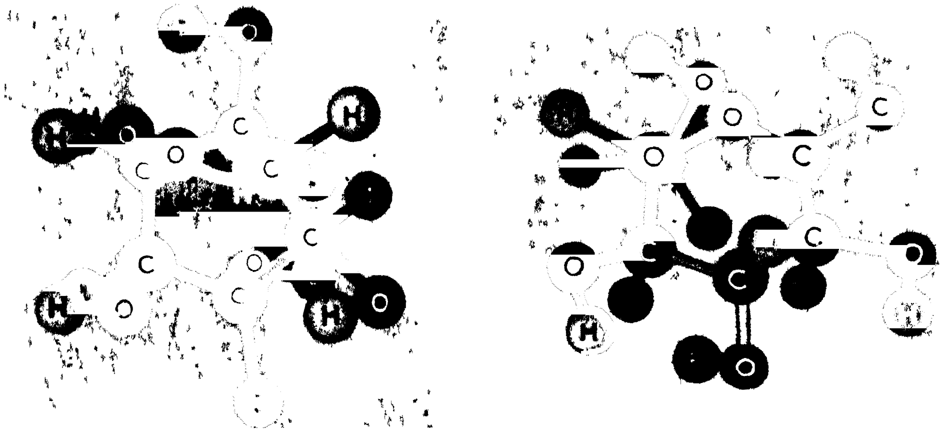
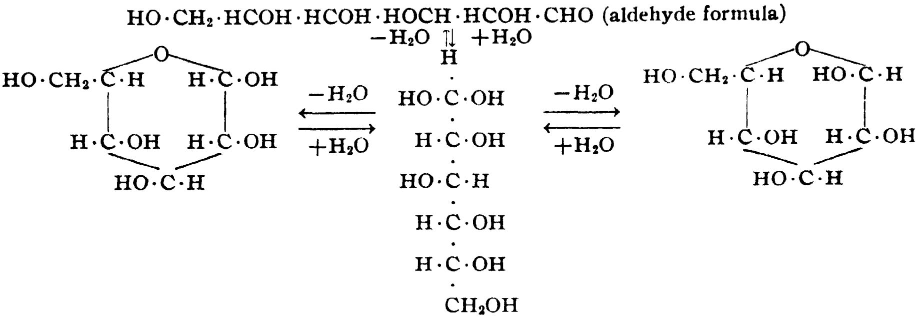

(*a*) (*b*)

Fig 12 Tinker toy models of *L* glucose in the continuous chain and pyranoid forms (*a*) This illustration shows the tendency of the molecule to spiral (*b*) The pyranoid ring of *L* glucose as the consequence of a proton shift in the structure shown in Fig 12*a*.

D^+-Glucose, by this reasoning, has been shown to have an empirical formula of CH_2O, a molecular formula of $C_6H_{12}O_6$, a skeleton formula of $C \cdot C \cdot C \cdot C \cdot C \cdot C$, a semiconstitutional formula of $C_6H_7O(OH)_5$, a constitutional formula of $HO \cdot CH_2(CHOH)_4 \cdot CHO$, and a semistructural formula of $HO \cdot CH_2 \cdot HCOH \cdot HCOH \cdot HOCH \cdot HCOH \cdot CHO$. Its structure in aqueous solution is best represented by the following equilibrium.[15]

---

[15] See Ed F. Degering *et al*, *Outline of Organic Chemistry* Barnes and Noble, New York, 1941, pp 105-110.



$HO \cdot CH_2 \cdot HCOH \cdot HCOH \cdot HOCH \cdot HCOH \cdot CHO$ (aldehyde formula)

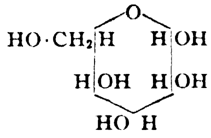
α-*D*-Glucose, $[\alpha]^{20}_{D} = +112.2$ (pyranoid ring formula)

(Aldehydrol formula)

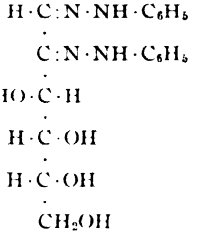
β-*D*-Glucose, $[\alpha]^{20}_{D} = +18.7$ (pyranoid ring formula)

For this equilibrium mixture, $[\alpha]^{20}_{D} = 52.7$.

The facts which necessitate a postulated equilibrium of this general type are (*1*) the existence of two separate and distinct forms of D^+-glucose, that is, α-D^+-glucose and β-D^+-glucose, (*2*) the comparative ease with which one hydrogen atom is replaced in glucoside formation, (*3*) the possibility of obtaining more than one α-methyl-D^+-glucoside by varying the conditions of alkylation, and (*4*) the tendency for either α-D^+-glucose or β-D^+-glucose to undergo a rapid change in its rotatory power when dissolved in water because of a shift toward an equilibrium, which is described as mutarotation.

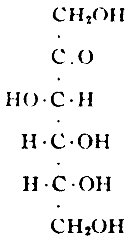


α-D^+-Glucopyranose



$$\begin{array}{l} H \cdot C{:}N \cdot NH \cdot C_6H_5 \\ C{:}N \cdot NH \cdot C_6H_5 \\ HO \cdot C \cdot H \\ H \cdot C \cdot OH \\ H \cdot C \cdot OH \\ CH_2OH \end{array}$$

Glucosazone



$$\begin{array}{l} CH_2OH \\ C{:}O \\ HO \cdot C \cdot H \\ H \cdot C \cdot OH \\ H \cdot C \cdot OH \\ CH_2OH \end{array}$$

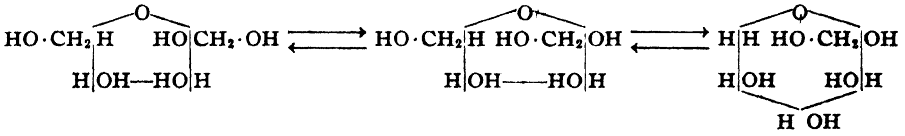
D^--Fructose

Helferich[16] and various other workers have obtained experimental evidence to the effect that the stable form of D^+-glucose, both free and combined, is best represented by the δ- or pyranose ring structure shown at the upper right.

Once the structure of D^+-glucose is established, that of many of the other monosaccharides may be determined by analogy. It is known, for example, that the reduction of D^--fructose gives D^--sorbitol and D^+-mannitol, and that D^--fructose, D^+-glucose, and D^+-mannose all yield the same osazone. Since osazone formation involves only the two terminal carbon groupings, as represented by the osazone formula shown at the right, it is apparent that the lower four carbon groupings in D^--fructose, D^+-glucose, and D^+-mannose must be identical. The fact that D^--fructose is a ketose sugar, together with the establishment of the lower four groupings as identical with those in D^+-glucose, makes it imperative that the keto group be assigned to position 2 and that the semistructural formula for D^--fructose be written as indicated at the right. It has been shown experimentally that fructose as a building unit of sucrose exists in the γ- or *furanoid*-ring structure, but in the uncombined form it exists principally in the δ- or pyranoid-ring structure:

---

[16] Ed. F. Degering *et al.*, *Outline of Organic Chemistry.* Barnes and Noble, New York, 1941, p. 110. H. Gilman, *Organic Chemistry for Advanced Students.* Wiley, New York, 1938, p. 1421.



α-γ-D^--Fructose[17] (furanoid ring)

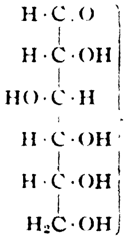
β-γ-D^--Fructose (furanoid ring)

β-Δ-D^--Fructose (pyranoid ring)

The structural relationship among the common monosaccharides is shown by the following formulas:

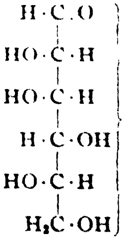
	CHO	CH₂OH	CHO	CHO	
CHO	H·C·OH	C:O	HO·C·H	H·C·OH	CHO
HO·C·H	HO·C·H	HO·C·H	HO·C·H	HO·C·H	*H*·C·*OH*
H·C·OH	H·C·OH	H·C·OH	H·C·OH	*HO*·C·H	*HO*·C·H
H·C·OH	H·C·OH	H·C·OH	H·C·OH	H·C·OH	H·C·OH
CH₂OH	CH₂OH	CH₂OH	CH₂OH	CH₂OH	CH₂OH
D^--Arabinose	D^+-Glucose	D^--Fructose	D^+-Mannose	D^+-Galactose	D^+-Xylose

Some interconversion and structural relationships among the monosaccharides may be shown by:



D^+-Glucose is

(1) the mirror image of L^--glucose.
(2) the epimer of D^+-mannose.
(3) degraded by the Wohl process to give D^--arabinose.
(4) changed by inversion into L^+-gulose.
(5) obtained from D^--arabinose by the Kiliani synthesis.



L^+-Gulose is

(1) the mirror image of D^--gulose.
(2) the epimer of L^+-idose.
(3) degraded by the Wohl process to give L^--xylose.
(4) changed by inversion into D^+-glucose.
(5) obtained from L^--xylose by the Kiliani synthesis.

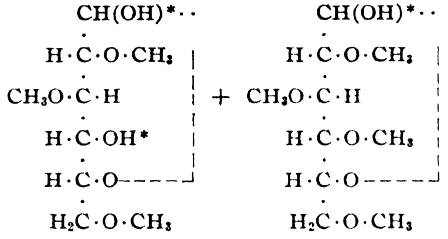
---

[17] In the combined form, levulose occurs as a furanoid ring, but in the free state, it doubtless exists as the pyranoid or six-numbered ring.

IV. STRUCTURE OF DISACCHARIDES

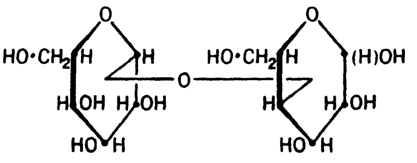
The structures of the disaccharides are established by determining (*1*) the component monosaccharide units, (*2*) the specific oxygen bridge in each building unit, (*3*) the particular carbon atoms involved in the glycosidic linkage, and (*4*) the specific isomeric form (α- or β-) of one or both units. In the case of maltose, for example, the complete methylation and subsequent controlled hydrolysis gives 2,3,6-trimethylglucose and 2,3,4,6-tetramethylglucose, from which data it is seen that the component units are glucose residues. In the case of the 2,3,4,6-tetramethylglucose, the only possible oxide structure is the 1,5- or the stable pyranoid structure, leaving the 1-position open for linkage to the other glucose unit. The presence of the pyranoid ring structure in the 2,3,6-trimethylglucose residue can be established either by progressive degradation of maltose, from which it is observed that the intermolecular linkage is bonded to carbon atom 4, or by analogy to other compounds that contain the pyranoid structure. By either method of attack, it may be shown that the pyranoid ring exists in each of the component glucose units. The facts that maltose is a reducing sugar, and that only one 4-position and two 1-positions are open in the residual hydrolytic units, clearly indicate linkage of the 4-glucosyl type. The specific isomeric form is determined by the use of enzymes, the *maltase* hydrolyzing what has been defined as an α-linkage whereas *emulsin* attacks a β-linkage.[18] In the case of maltose, enzymatic treatment indicates that it is an α-glucosidic linkage. It becomes possible, then, to write the name of maltose as 4-α-D^+-glucosyl-D^+-glucose. The evidence at present indicates that the best formulations of the more important disaccharides are:

Maltose + complete methylation + controlled hydrolysis ⟶

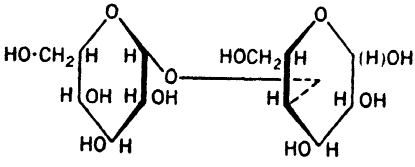


2,3,6-Trimethylglucose 2,3,4,6-Tetramethylglucose

* Points of possible linkage between the two units are shown by the asterisks.



Cellobiose, 4-β-D^+-glucosyl-D^+-glucose

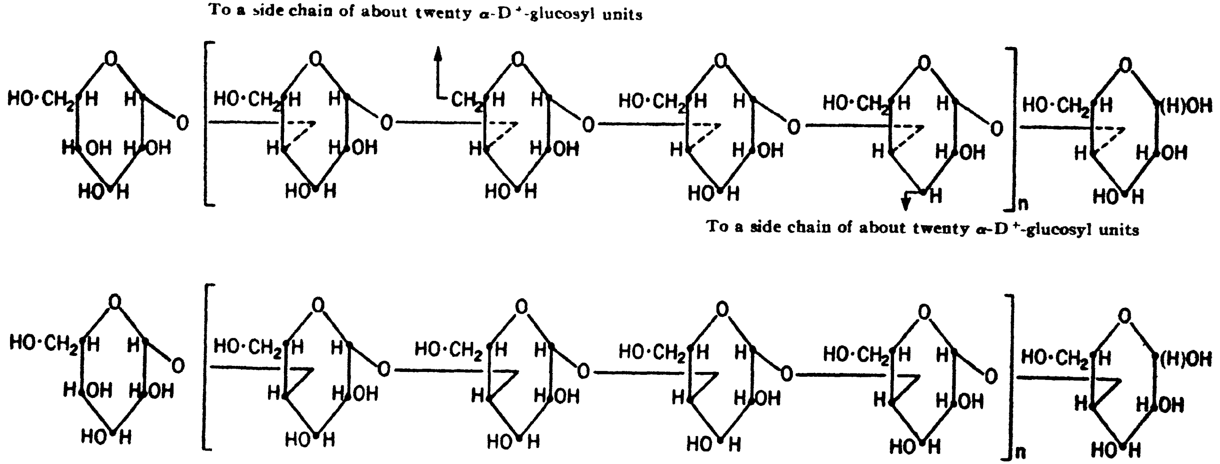


Maltose, 4-α-D^+-glucosyl-D^+-glucose

---

[18] This fact was used initially to establish the specificity of enzymes, but by definition **a glycosidic linkage** which is hydrolyzed by the enzyme maltase is an α-linkage.

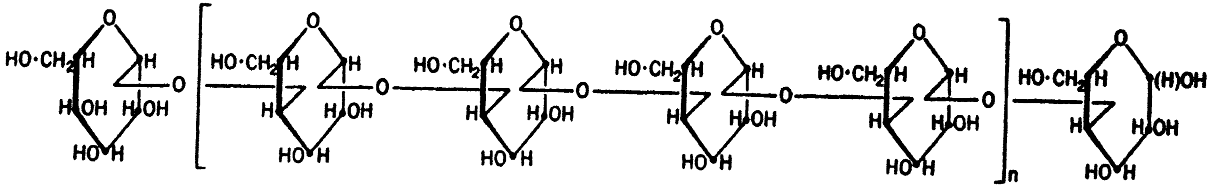
Building units of the starch molecule.



To a side chain of about twenty α-D⁺-glucosyl units

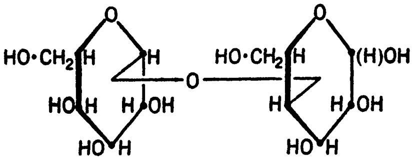
To a side chain of about twenty α-D⁺-glucosyl units

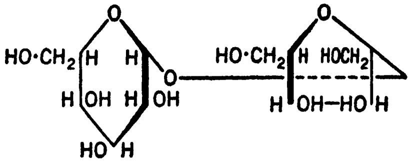
Building unit of the cellulose molecule.



Cellulose is predominantly of the continuous chain variety (lower formula), whereas most of the starches contain about 30% of the straight chain variety (middle formula) and 70% of the branched variety (upper formula).

These formulas[19] indicate that maltose may be designated as 4-α-glucosylglucose, cellobiose as 4-β-glucosylglucose, lactose as 4-β-galactosylglucose, and sucrose as α-glucosyl-β-γ-fructoside. It is understood that the D^+-form of glucose and the D^--form of fructose are the building units concerned.


Lactose, 4-β-D^+-galactosyl-D^+-glucose


Sucrose, α-D^+-glucosyl β-γ-D^--fructoside

As the result of an enormous amount of research on the structure of the starch and cellulose molecules it is now generally accepted that the essential building units of these two carbohydrate materials are those shown on page 63.

V. NOMENCLATURE

Common names predominate, although systematic names have been proposed. D^+-Glucose, for example, may be named as pentahydroxyhexanal $^{+-++}$, the plus and minus indicating that when the formula of D^+-glucose is oriented so that the aldehyde group is at the top, the right-hand substituents for the α-, β-, γ-, and δ-carbon groupings are OH, H, OH, OH, as indicated at the right.

H·C:O
H·C·*OH* or +
HO·C·*H* or −
H·C·*OH* or +
H·C·*OH* or +
CH_2OH
D^+-Glucose

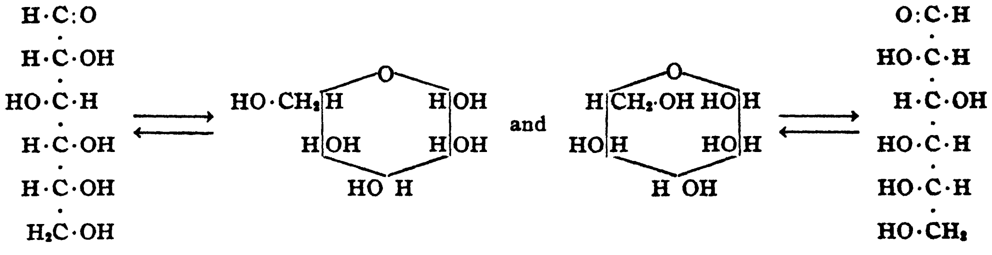
The work of Wohl on glyceraldehyde makes it possible to relate all of the sugars structurally to D^+- and L^--glyceraldehyde. If, accordingly, the formula of a sugar is oriented so that the aldehyde group is at the top, the relative position of the *OH* group on the lower asymmetric carbon grouping establishes the family relationship. If the δ-*OH* group in the *hexose* series is on the right, the sugar belongs to the *D*-series, but if the δ-*OH* group is on the left, the sugar belongs to the *L*-series. If a D^+-sugar is dextrorotatory and an L^--sugar is levorotatory, these designations appear to be satisfactory. If, however, a *D*-sugar is levorotatory, it can be conveniently indicated by the use of the symbol D^-- which connotes that a member of the *D*-series is levorotatory. Similarly, L^+- is taken to mean that a member of the *L*-series is dextrorotatory. Various other symbols have been used.

Ring formation occurs *by the migration of the hydrogen atom of the hydroxyl group in the 5-position to the carbonyl group* (see page 66, showing lactol formation).

---

[19] In writing formulas by this convention, atoms written to the right of the point of attachment in both the *D*- and the *L*- series lie *below* the plane of the molecule, and atoms written to the left of the point of attachment lie *above* the plane of the molecule. The use of (H)OH to the right of the point of attachment indicates a *mixture* of both forms.

The relationship between the straight chain and ring formula is indicated by the following formulas:

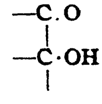


D^+-Glucose α-D^+-Glucose α-L^--Glucose L^--Glucose

In the ring structure of α-D^+-glucose, *all of the groups written to the left are above the plane of the molecule*, and in the corresponding formula for L^--glucose, all of the groups written to the left are also *above* the plane of the molecule.

The polyhydroxy aldehydes are designated as aldoses whereas the polyhydroxy ketones are ketoses. The term *aldotetrose* indicates a polyhydroxy aldehyde with a four carbon content which in I. U. C. terminology would be a 2,3,4-trihydroxybutanal, the *aldo-* indicating the aldehydic function and the *tetrose* designating the carbon content of the molecule. Contrasted to this is ketotetrose, or a 1,3,4-trihydroxybutanone, the *keto-* suggesting the ketonic function and the *tetrose* indicating the number of carbon atoms in the molecule.

The carbohydrates, in general, have names that end in *ose*. The characteristic functional group, sometimes designated as the *ose*-group, which is present, actually or potentially, in all carbohydrate material is indicated at the right. By reduction of the carbonyl group in the simple sugars the corresponding polyhydroxy alcohols are obtained, whose names are characterized by the ending *itol*, arabinose becoming arabitol, mannose becoming mannitol, and glucose and sorbose becoming sorbitol. If the carbonyl group is oxidized to the corresponding polyhydroxy acid, the name of the product is characterized by the ending *onic* acid, glucose becoming gluconic acid, mannose becoming mannonic acid, and arabinose becoming arabinonic acid.

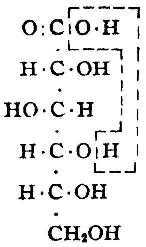


$$\begin{array}{l} -\mathrm{C.O} \\ \quad | \\ -\mathrm{C{\cdot}OH} \\ \quad | \end{array}$$

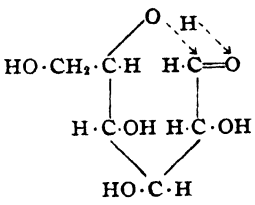
The *ose*-group

The *uronic acids* are derivatives of the simple sugars in which only the primary alcohol group ($\cdot CH_2 \cdot OH$) has been oxidized to a carboxyl group ($\cdot CO_2H$), the aldehyde group having been protected by glycoside formation.

The lactones are the products formed by the loss of an equivalent of water from the carboxyl group and some one of the alcoholic hydroxyl groups, usually from the γ-hydroxyl group, to yield a γ-lactone. D^+-Gluconic acid, for example, readily loses an equivalent of water to give γ-D^+-gluconolactone. Closely related to the formation of the lactones, by the loss of an equivalent of water, is the formation of the lactols (or lactals or hemiacetals) by the migration of the hydrogen atom from the *δ-OH* group to the carbonyl oxygen atom with the consequent formation of a δ- or pyranose oxide ring structure as indicated by the following:



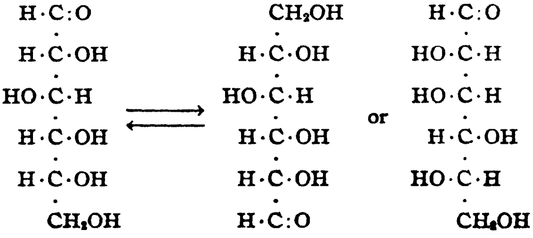
Showing lactone formation



Showing lactol formation

Although the δ-lactols are the stable forms, experimental evidence points to the fact that the fructose unit in sucrose actually exists in the γ- or furanoid ring form. In the uncombined form, D^--fructose probably exists almost entirely in the form of the pyranoid or δ-oxide ring structure. This type of structure is also designated as a semiacetal or hemiacetal.

The term *inversion*[20] applies to a series of changes by which the carbonyl group of an aldose is reduced to a primary alcohol group ($\cdot CHO$ is reduced to $\cdot CH_2\cdot OH$) and the initial primary alcohol group is oxidized to a carbonyl group ($\cdot CH_2\cdot OH$ is oxidized to $\cdot CHO$). The same effect is obtained if the asymmetric nucleus is rotated through 180° in the plane of the paper.



$$\begin{array}{c} H\cdot C:O \\ H\cdot C\cdot OH \\ HO\cdot C\cdot H \\ H\cdot C\cdot OH \\ H\cdot C\cdot OH \\ CH_2OH \\ \text{I} \end{array} \rightleftarrows \begin{array}{c} CH_2OH \\ H\cdot C\cdot OH \\ HO\cdot C\cdot H \\ H\cdot C\cdot OH \\ H\cdot C\cdot OH \\ H\cdot C:O \\ \text{II} \end{array} \quad \text{or} \quad \begin{array}{c} H\cdot C:O \\ HO\cdot C\cdot H \\ HO\cdot C\cdot H \\ H\cdot C\cdot OH \\ HO\cdot C\cdot H \\ CH_2OH \\ \text{III} \end{array}$$

I II III

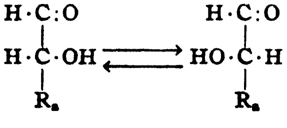
Illustrating inversion

In the three formulas shown, formula II is obtained from formula I by reduction of the carbonyl group to a primary alcohol group and the oxidation of the primary alcohol group to a carbonyl group. By rotating formula II through 180° in the plane of the paper, formula III is obtained. Formula III differs from formula I in that the asymmetric nucleus has been rotated 180° in the plane of the paper.

Epimeric change describes the transposition of the *H* and *OH* groups of the α-carbon grouping, as indicated below. The change is effected chemically by digesting the polyhydroxy acid in pyridine for a number of hours to give an equilibrium mixture. This is separated by use of brucine or some other appropriate alkaloid, and the isolated salts then converted to the free acids, which automatically yield the lactones. The lactones are reduced in turn to the same or an epimeric aldose. This is a special case of Walden inversion.[21]

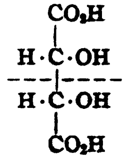
---

[20] This term (inversion) should not be confused with the hydrolysis of sucrose which is technically referred to as inversion because a change of rotation accompanies hydrolysis.



$$\begin{array}{c}\mathrm{H\cdot C{:}O}\\ |\\ \mathrm{H\cdot C\cdot OH}\\ |\\ \mathrm{R_a}\end{array} \rightleftarrows \begin{array}{c}\mathrm{H\cdot C{:}O}\\ |\\ \mathrm{HO\cdot C\cdot H}\\ |\\ \mathrm{R_a}\end{array}$$

Illustrating epimeric change



$$\begin{array}{c}\mathrm{CO_2H}\\ |\\ \mathrm{H\cdot C\cdot OH}\\ \text{---|----}\\ \mathrm{H\cdot C\cdot OH}\\ |\\ \mathrm{CO_2H}\end{array}$$

Illustrating internal compensation

Internal compensation describes the phenomena in which the optical activity of one half of a molecule counteracts that of the other half of the molecule. Such compensation results when a molecule is symmetrical with respect to a point, to a line, to a plane, or to a rotating axis of symmetry. If half of the molecule is the mirror image of the other half, the molecule is symmetrical and will be internally compensated and optically inactive.

Mutarotation describes the shift toward equilibrium whenever an α- or a β-form of a sugar is dissolved in water. The optical activity of the resulting mixture will have a value intermediate between that of the two forms, the exact value being determined by the point of equilibrium.

1. Classification

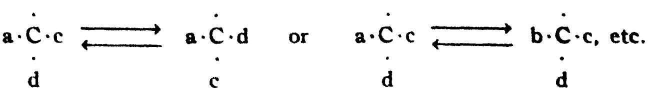
A classification of the carbohydrates will aid in the clarification of carbohydrate nomenclature. The usual classification of the more common members, which is based upon composition and structure, is given.

I. Monosaccharides

- (*a*) Diose (two carbon atoms), as $HO\cdot CH_2\cdot CHO$, glycolaldehyde.
- (*b*) Trioses (three carbon atoms), which are subdivided into
  - (1) Aldotrioses, as $HO\cdot CH_2\cdot HCOH\cdot CHO$, D^+-glyceraldehyde, and
  - (2) Ketotriose, as $HO\cdot CH_2\cdot CO\cdot CH_2\cdot OH$, dihydroxyacetone.
- (*c*) Tetroses (four carbon atoms), which are subdivided into
  - (1) Aldotetroses,
  - (2) Ketotetroses, and
  - (3) Desoxyaldotetroses.

---

[21] Walden inversion describes the interchange of any two substituents of an asymmetric center as indicated below:



$$\begin{array}{c}\mathrm{b}\\ \cdot\\ \mathrm{a\cdot C\cdot c}\\ \cdot\\ \mathrm{d}\end{array} \rightleftarrows \begin{array}{c}\mathrm{b}\\ \cdot\\ \mathrm{a\cdot C\cdot d}\\ \cdot\\ \mathrm{c}\end{array} \quad \text{or} \quad \begin{array}{c}\mathrm{b}\\ \cdot\\ \mathrm{a\cdot C\cdot c}\\ \cdot\\ \mathrm{d}\end{array} \rightleftarrows \begin{array}{c}\mathrm{a}\\ \cdot\\ \mathrm{b\cdot C\cdot c,\ etc.}\\ \cdot\\ \mathrm{d}\end{array}$$

(*d*) Pentoses (five carbon atoms), which are subdivided into
  (1) Aldopentoses, as $HO\cdot CH_2\cdot HCOH\cdot HCOH\cdot HOCH\cdot CHO$, D^--arabinose, $HO\cdot CH_2\cdot HCOH\cdot HOCH\cdot HCOH\cdot CHO$, D^+-xylose, $HO\cdot CH_2\cdot HCOH\cdot HCOH\cdot HCOH\cdot CHO$, D^--ribose, the three most important members,
  (2) Ketopentoses, and
  (3) Desoxyaldopentoses.
(*e*) Hexoses (six carbon atoms)[22] which are subdivided into
  (1) Aldohexoses, as D^+-glucose, D^+-galactose, and D^+-mannose, the three most important members, whose structures have been considered on page 61,
  (2) Ketohexoses, as $HO\cdot CH_2\cdot HCOH\cdot HCOH\cdot HOCH\cdot CO\cdot CH_2OH$, D^--fructose, and
  (3) Desoxyaldohexoses as rhamnose.
(*f*) Glycosides which are subdivided into the di-, tri-, and polysaccharides and the simple glycosides as methyl α-D^+-glucoside. Glucoside formation is utilized by the animal organism for the elimination of certain toxic materials.

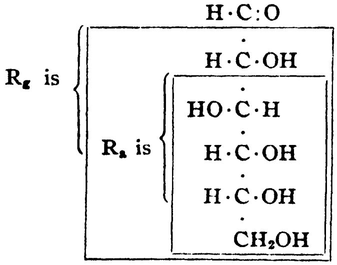
II. Disaccharides

(*a*) Cellobiose, or 4-β-glucosylglucose,
(*b*) Lactose, milk sugar, or 4-β-galactosylglucose,
(*c*) Maltose, malt sugar, or 4-α-glucosylglucose, and
(*d*) Sucrose, cane sugar, or α-glucosyl-β-γ-fructoside.

III. Polysaccharides

(*a*) Pentosans, the anhydrides of the pentoses, as illustrated by the
  (1) Arabinosans, or arabans, which occur in cherry and mesquite gum, and
  (2) Xylosans, or xylans, which occur in bran, corncobs, and woody materials.
(*b*) Hexosans, the anhydrides of hexoses, of which the more important types are
  (1) Dextrans, the anhydrides of dextrose or glucose, which have four important representatives: the dextrins, the glycogens, the starches, and the celluloses,
  (2) Galactosans, or galactans, the anhydrides of galactose, which are sparsely distributed,
  (3) Levulosans, or levulans, the anhydrides of levulose, which are found in chicory root, dahlia bulbs, dandelion roots, and Jerusalem artichokes, and
  (4) Mannosans, or mannans, which are obtained from vegetable ivory. The vegetable ivory is obtained from a nut grown principally in South America and is used for buttons.

In the subsequent discussion of the methods of preparation and the chemical properties it will be convenient to condense formulas as much as clarity will permit. This can be done by employing the use of the radicals shown below whenever the reaction under consideration involves the activity of only the carbonyl group. The R_a indicates an arabinose residue whereas the R_g represents a glucose residue.



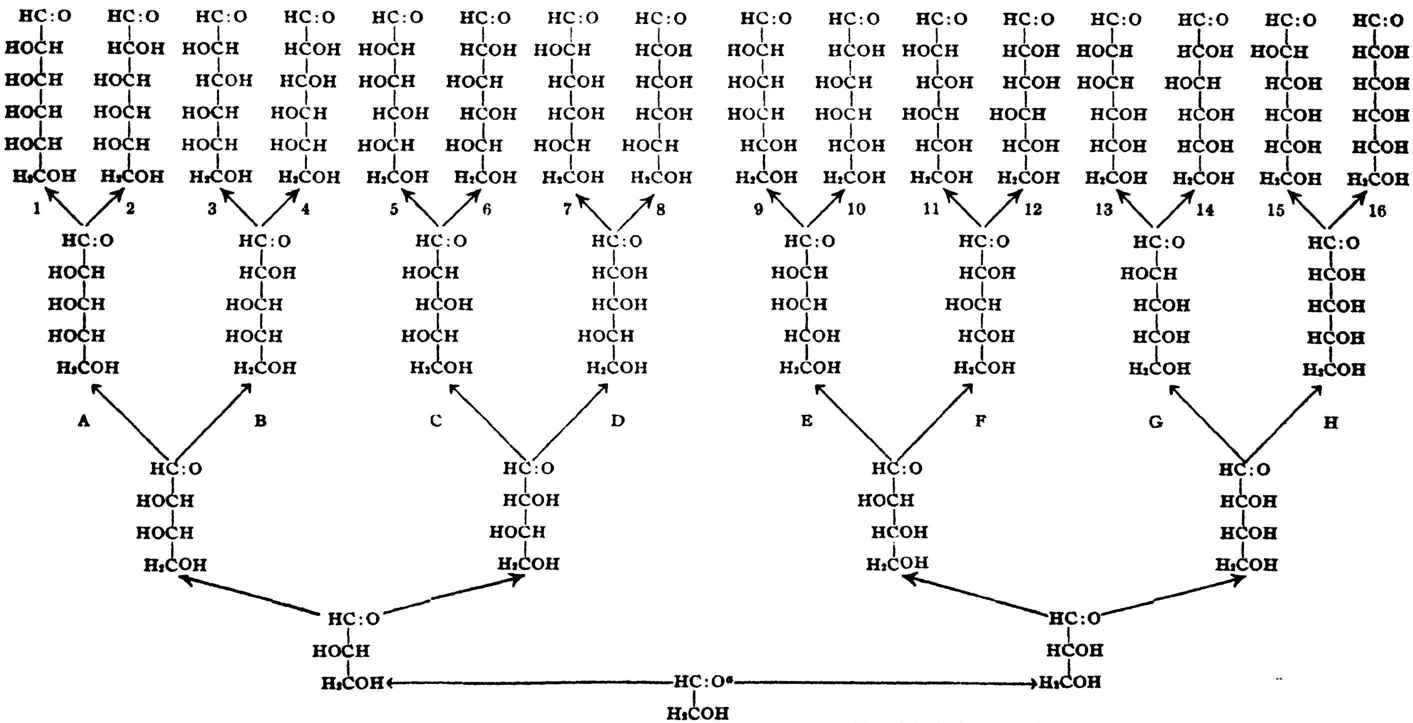
$$\begin{array}{r l} & H\cdot C{:}O \\ & \quad \cdot \\ R_g \text{ is } & \left\{ \boxed{\begin{array}{r l} & H\cdot C\cdot OH \\ & \quad \cdot \\ R_a \text{ is } & \left\{ \boxed{\begin{array}{c} HO\cdot C\cdot H \\ \cdot \\ H\cdot C\cdot OH \\ \cdot \\ H\cdot C\cdot OH \\ \cdot \\ CH_2OH \end{array}} \right. \end{array}} \right. \end{array}$$

---

[22] Fischer, by the Kiliani synthesis, made glucoheptose, glucooctose, and gluconnoose, and Philippe synthesized glucodecose in 1912.

TABLE 3*a*

MODIFIED ROSANOFF CLASSIFICATION CHART



Kiliani synthesis:
^{a} $HOCH_2{\cdot}CHO + H{\cdot}CN/(\text{trace } NH_3) + 2H_2O/H^+ - H_2O + 2H_2O/2Na{\cdot}Hg/2H^+$, aq. $\longrightarrow$ $HO{\cdot}CH_2{\cdot}HCOH{\cdot}CHO$ and $HO{\cdot}CH_2{\cdot}HOCH{\cdot}CHO$

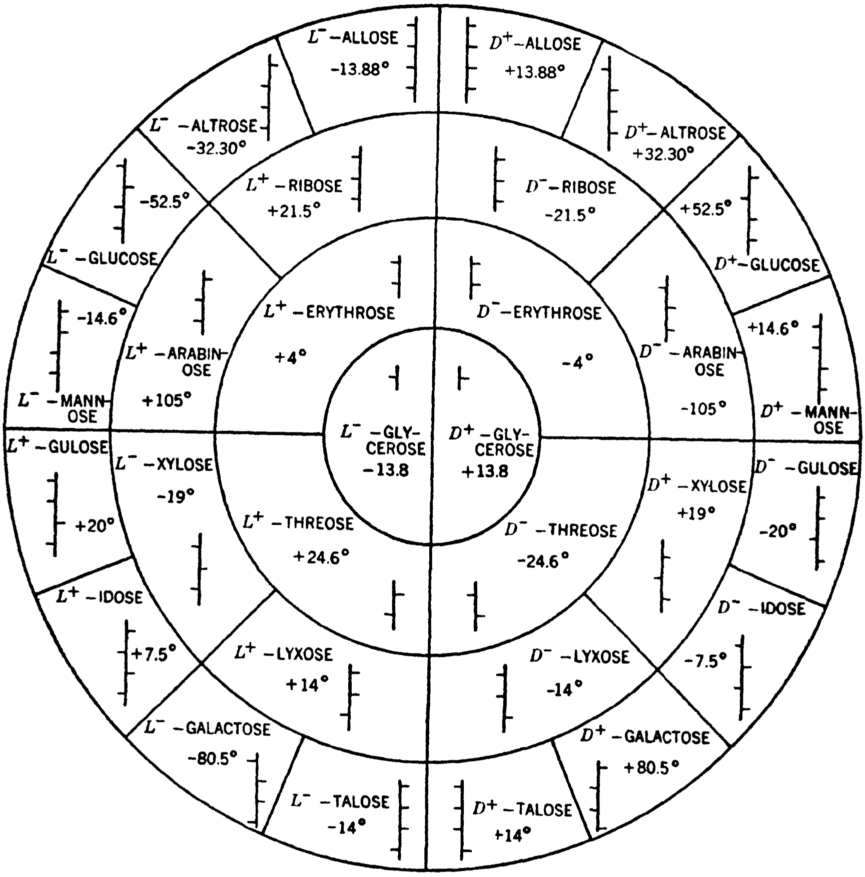
Although saccharin is frequently associated with the carbohydrates because of its pronounced sweetness, it is structurally unrelated; saccharin is the imide of *o*-sulfobenzoic acid. It was discovered by Ira Remsen, is several hundred times as sweet as sugar, and is used to a limited extent as a substitute for sugar in the treatment of diabetic patients. It has no food value and so far as is known exerts no injurious effect upon the animal organism.

2. System of the Aldohexoses

The configuration of the simple aldohexoses, their relationship, and their optical activity are shown in the modified Rosanoff Classification Chart (Tables 3*a* and *b*).

TABLE 3*b*

ROSANOFF CLASSIFICATION CHART

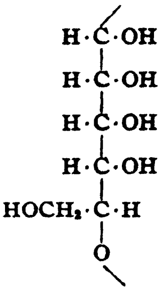
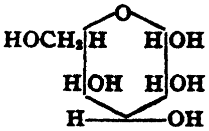
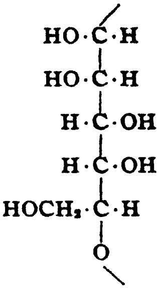
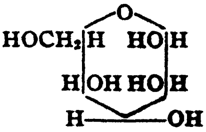
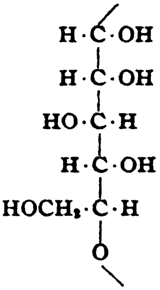
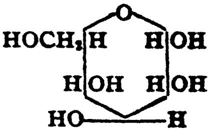
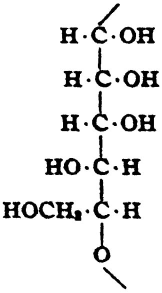
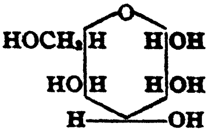


L-Configuration
Levorotatory property is indicated by a minus exponent.

D-Configuration
Dextrorotatory property is indicated by a plus exponent.

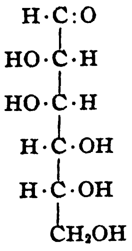
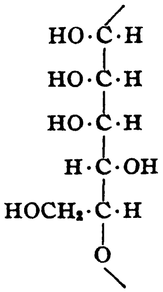
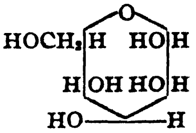
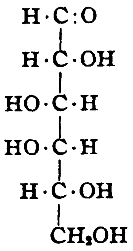
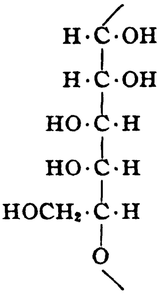
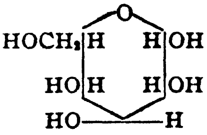
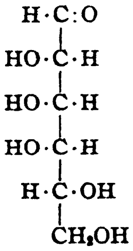
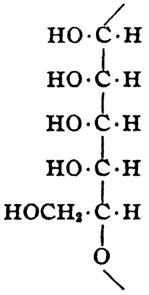
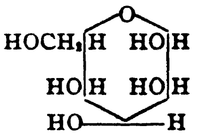
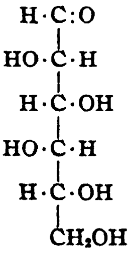
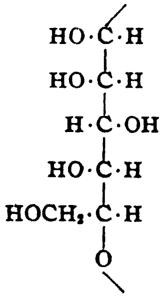
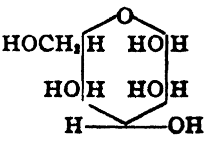
TABLE 4

α-*D*-SERIES OF THE HEXOPYRANOSES

Sugar	Rosanoff notation	Projection formula	Proposed notation	Projection formula	Space formula
α-*D*-Allose	++++	H·C:O H·C·OH H·C·OH H·C·OH H·C·OH CH_2OH	+++++		
α-*D*-Altrose	−+++	H·C:O HO·C·H H·C·OH H·C·OH H·C·OH CH_2OH	−−+++		
α-*D*-Glucose	+−++	H·C:O H·C·OH HO·C·H H·C·OH H·C·OH CH_2OH	++−++		
α-*D*-Gulose	++−+	H·C:O H·C·OH H·C·OH HO·C·H H·C·OH CH_2OH	+++−+		

(*Continued on page 72*)

TABLE 4 (*Continued*)
α-D-SERIES OF THE HEXOPYRANOSES

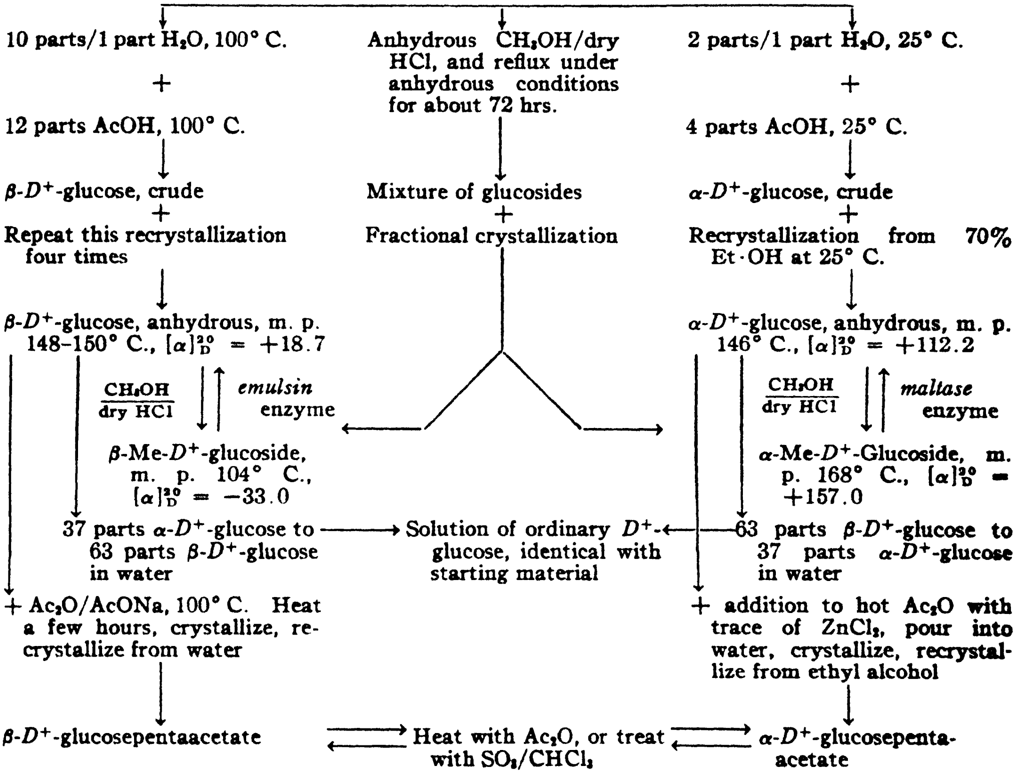
Sugar	Rosanoff notation	Projection Formula	Proposed notation	Projection formula	Space formula
α-D-Mannose	−−++	 $\begin{array}{c} \mathrm{H\cdot C{:}O} \\ \mathrm{HO\cdot C\cdot H} \\ \mathrm{HO\cdot C\cdot H} \\ \mathrm{H\cdot C\cdot OH} \\ \mathrm{H\cdot C\cdot OH} \\ \mathrm{CH_2OH} \end{array}$	−−−++		
α-D-Galactose	+−−+	 $\begin{array}{c} \mathrm{H\cdot C{:}O} \\ \mathrm{H\cdot C\cdot OH} \\ \mathrm{HO\cdot C\cdot H} \\ \mathrm{HO\cdot C\cdot H} \\ \mathrm{H\cdot C\cdot OH} \\ \mathrm{CH_2OH} \end{array}$	++−−+		
α-D-Talose	−−−+	 $\begin{array}{c} \mathrm{H\cdot C{:}O} \\ \mathrm{HO\cdot C\cdot H} \\ \mathrm{HO\cdot C\cdot H} \\ \mathrm{HO\cdot C\cdot H} \\ \mathrm{H\cdot C\cdot OH} \\ \mathrm{CH_2OH} \end{array}$	−−−−+		
α-D-Idose	−+−+	 $\begin{array}{c} \mathrm{H\cdot C{:}O} \\ \mathrm{HO\cdot C\cdot H} \\ \mathrm{H\cdot C\cdot OH} \\ \mathrm{HO\cdot C\cdot H} \\ \mathrm{H\cdot C\cdot OH} \\ \mathrm{CH_2OH} \end{array}$	−−+−+		

The α-*D*-series of the hexosepyranoses, together with the Rosanoff notation, the projection formula, the proposed notation, its projection formula, and the space formula, are shown in Table 4. The interrelationships of the α- and β-forms of D^+-glucose are shown in Table 5.

TABLE 5

THE ALPHA, BETA-D^+-GLUCOSE SYSTEM

Crystalline D^+-glucose, variable m. p., $[\alpha]_D^{20} = +52.7$


10 parts/1 part H_2O, 100° C.
+
12 parts AcOH, 100° C.
β-D^+-glucose, crude
+
Repeat this recrystallization four times
β-D^+-glucose, anhydrous, m. p. 148–150° C., $[\alpha]_D^{20} = +18.7$
$\frac{CH_3OH}{dry\ HCl}$
emulsin enzyme
β-Me-D^+-glucoside, m. p. 104° C., $[\alpha]_D^{20} = -33.0$
37 parts α-D^+-glucose to 63 parts β-D^+-glucose in water
+ Ac_2O/AcONa, 100° C. Heat a few hours, crystallize, recrystallize from water
β-D^+-glucosepentaacetate
Anhydrous CH_3OH/dry HCl, and reflux under anhydrous conditions for about 72 hrs.
Mixture of glucosides
+
Fractional crystallization
Solution of ordinary D^+-glucose, identical with starting material
Heat with Ac_2O, or treat with $SO_2/CHCl_3$
2 parts/1 part H_2O, 25° C.
+
4 parts AcOH, 25° C.
α-D^+-glucose, crude
+
Recrystallization from 70% Et·OH at 25° C.
α-D^+-glucose, anhydrous, m. p. 146° C., $[\alpha]_D^{20} = +112.2$
$\frac{CH_3OH}{dry\ HCl}$
maltase enzyme
α-Me-D^+-Glucoside, m. p. 168° C., $[\alpha]_D^{20} = +157.0$
63 parts β-D^+-glucose to 37 parts α-D^+-glucose in water
+ addition to hot Ac_2O with trace of $ZnCl_2$, pour into water, crystallize, recrystallize from ethyl alcohol
α-D^+-glucosepentaacetate

It should be observed that the direction of rotation is consistent for the trioses, that all of the tetroses are abnormal in this respect, that the pentoses are all abnormal except the xyloses, and that the hexoses are all normal except the idoses and guloses.

VI. COMMERCIAL SOURCES

1. Controlled Hydrolysis of Starch

(a) Preparation of Corn Sirup

A measured quantity of acid (60 to 70 lbs. of 18 Baumé hydrochloric acid) in water is added to a pressure vessel. To this is added about 2000 gallons of a starch slurry (containing about 8000 lbs. of starch) of 22 or more Baumé, during violent agitation with steam, over a period of 7 to 10 mins. By this procedure the starch is simultaneously pasted and liquefied. The vessel is then closed and subjected to 35 lbs. pressure with live steam. Satisfactory conversion is generally obtained in about four minutes. This may be checked by colorimetric iodine tests on samples withdrawn from the converter. The product is neutralized, concentrated, bleached, and marketed as commercial corn sirup at 41, 42, or 43 Baumé.

(*b*) *Preparation of Sugar*

The sugar conversion is similar to that used in the production of corn sirup, except that a maximum conversion is desired. This is accomplished by use of slightly more acid in an appreciably more dilute solution of the starch. One commercial plant uses 70 lbs. of 18 Baumé hydrochloric acid per 2000 gal. of 10 Baumé starch slurry. Maximum conversion is obtained in 22 to 25 mins. at a pressure of 45 lbs. per square inch. Under these conditions a reducing substance content of about 91 to 92% is obtained, which is substantially the equilibrium mixture, and the dextrose content reaches about 86%. The hydrolyzed product, in the case of both sirup and sugar, is neutralized with soda ash to a *p*H of 4.8 or thereabouts to remove insoluble protein, fatty acids, or other impurities, filtered, bleached either with activated vegetable carbon or bone char or some combination of the two, and is then concentrated in suitable evaporators (generally of the multiple effect type) to about 30 Baumé. The concentrate is further bleached and then evaporated to the desired concentration for commercial use. In commercial practice there are two alternatives: the concentrated liquor may be: (*1*) run onto tables, allowed to cool and solidify, and then sold as slab or chip sugar; or (*2*) seeded with dextrose crystals and cooled slowly under carefully regulated agitation until crystallization is complete, centrifuged, dried to the monohydrate, and marketed as commercial dextrose.

2. Isolation from Natural Products

Sucrose is obtained from sugar cane and sugar beets, each supplying about one-half of the annual consumption in the United States. The essential steps in the commercial preparation of sucrose from sugar cane are maceration, extraction, clarification, defecation, concentration, evaporation, crystallization, separation, and drying to give the raw sugar which is sent to the refineries for further purification. In the beet-sugar process, the beets are cut into V-shaped slices (cosettes or noodles) which are extracted by countercurrent hot water to give about a 12% sucrose solution. The subsequent treatment of the solution is approximately the same as in the sugar-cane process. These processes are described in detail in Volume II, Chapter XVII. The pressed cane residue, bagasse, from the sugar cane is used to manufacture Celotex, which is an insulating material.

The extraction of sugar from beets was first accomplished at the beginning of the

19th century. The process then introduced was about 3% efficient. In 1828 in France and in 1836 in Germany, good sugar was successfully produced from beets. In the United States the beet-sugar industry has assumed commercial proportions in the states of California, Colorado, Indiana, Kansas, Michigan, Montana, Nebraska, South Dakota, Utah, and Wisconsin, and is produced on a smaller scale in some of the other states. Raw sugar is imported into this country, under quota, and is refined locally.

Lactose is a by-product of the cheese industry. The casein and the fat components of the milk are removed by precipitation with rennet and subsequent passage through filter presses, to give a filtrate which is *whey*. The whey is neutralized with calcium carbonate, clarified by filtration, and then processed by concentration, evaporation, and crystallization to obtain the lactose. On the basis of the whole milk the yield of lactose is about 2.3%.

Maltose is obtained to a limited extent from sprouted barley or from the partial hydrolysis of starch. The processing consists of extraction, clarification, concentration, crystallization, filtration, and drying.

Dextrin may be obtained by the controlled hydrolysis of starch with either acid or diastase. Complete hydrolysis by diastase yields maltose.

Glycogen is obtained by extraction of liver tissue, particularly that of rabbits.

Starch is produced in the United States almost exclusively from corn. The corn is steeped in water containing a trace of sulfurous acid, which has a germicidal effect, and then shredded in squirrel cage disintegrators, suspended in water, and agitated to float off the corn seed or germ. The suspension of gluten, hull, and starch is drawn off and passed into rotating cylinders covered with bolting cloth which retains the hulls but permits the gluten and starch particles to pass through with the water. The gluten-starch suspension is then passed slowly through long, slightly inclined, shallow troughs which act as settling tanks for the starch, the gluten overflowing with the water at the lower ends. For better grades, the settlings are washed, resettled, and dried.

The gluten is processed and marketed as gluten meal. The germs are collected, dried, and pressed to obtain corn oil. The pressed cake is marketed as cattle food.

In China the principal source of starch is rice, whereas in Germany most of the starch is obtained from potatoes. In tropical countries, sago and tapioca starches are important products.

3. Utilization of Vegetative Crops of Cellulose

The agricultural capacity of the United States to produce cotton, hemp, jute, straw, wood, and other vegetative crops of cellulose is almost unlimited in so far as our potential demand is concerned. The problem is not so much one of production as it is one of utilization. Aside from the better utilization of the cellulose material available, the development of the southern pine (slash pine) has afforded a method of producing in 8 to 10 years a crop of cellulose for wood pulp.

VII. LABORATORY AND SPECIAL SYNTHESES

1. Aldol Condensation

The reaction was discovered by Butlerow in 1861.

$$2H\cdot CHO/(\text{lime water}) \longrightarrow HOCH_2\cdot CHO, \text{ glycolaldehyde}$$

The glycolaldehyde will condense in turn with a second equivalent of formaldehyde to give a mixture of the glyceraldehydes:

$$HO\cdot CH_2\cdot CHO + H\cdot CHO/(\text{lime water}) \longrightarrow HO\cdot CH_2\cdot HCOH\cdot CHO + HO\cdot CH_2\cdot HOCH\cdot CHO,$$

or it will condense with itself to yield a tetrose:

$$HO\cdot CH_2\cdot CHO + HO\cdot CH_2\cdot CHO/(\text{lime water}) \longrightarrow HO\cdot CH_2\cdot CHOH\cdot CHOH\cdot CHO$$

This condensation is of historical interest because it was used by Emil Fischer in the production of the first synthetic sugar, *dl*-fructose, from *dl*-glyceraldehyde. The mild alkali seems to effect tautomerization of glyceraldehyde.

$$HO\cdot CH_2\cdot HCOH\cdot CHO/(\text{mild alkali}) \rightleftarrows HO\cdot CH_2\cdot CO\cdot CH_2\cdot OH$$

and then

$$HO\cdot CH_2\cdot HCOH\cdot CHO + H\cdot CHOH\cdot CO\cdot CH_2OH/(\text{lime water}) \longrightarrow dl\text{-}HO\cdot CH_2\cdot CHOH\cdot CHOH\cdot CHOH\cdot CO\cdot CH_2OH$$

One theory postulates that similar reactions may be involved in the photosynthetic process of nature. In support of this theory the proponents have cited the facts that plants do absorb carbon dioxide from the air and liberate oxygen, that they draw moisture from the soil, and that certain tropical species give a test for formaldehyde.

2. Kiliani Synthesis

The addition of hydrogen cyanide (*a*), the hydrolysis of the cyanohydrin with sulfuric acid (*b*), the separation of the isomers by treatment with active brucine (*c*), the liberation of the free aldonic acid (*d*), the loss of water to form the lactone (*e*), and the reduction of the lactone (*f*) to give the next higher aldose:

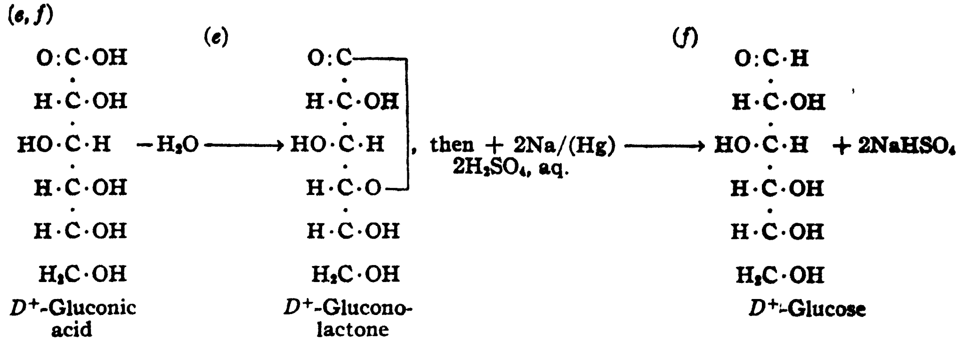
(*a*) $2R_aCHO + 2HCN/(\text{trace } NH_3), \text{ aq.} \longrightarrow R_a\cdot HCOH\cdot CN + R_a\cdot HOCH\cdot CN$, then

(*b*) $R_a\cdot HCOH\cdot CN, R_a\cdot HOCH\cdot CN + 4H_2O/2H_2SO_4 \longrightarrow R_a\cdot HCOH\cdot CO_2H, R_a\cdot HOCH\cdot CO_2H + 2(NH_4)_2SO_4$

(*c*) The mixture of the isomeric acids is then treated with active brucine and the brucine salts separated by fractional crystallization. The separated salts are treated with a base to precipitate the brucine, leaving a metallic salt of the sugar acid, such as $R_a\cdot HCOH\cdot CO_2Na$ in solution, then

(*d*) $R_a\cdot HCOH\cdot CO_2Na + H_2SO_4, \text{ aq.} \longrightarrow R_a\cdot HCOH\cdot CO_2H + NaHSO_4$, then

(e, f)



It should be observed that two isomers are always produced. In this case, starting with D^--arabinose (R_aCHO), the final products are D^+-glucose as given in step *f* and D^+-mannose which was not carried through steps *d*, *e*, and *f*.

It was the use of this reaction that enabled Kiliani to prove the skeleton formula of D^+-glucose and show it to be an aldose sugar. The reaction was of inestimable value to Emil Fischer in establishing the structures of the simple sugars.

3. Wohl Degradation Reaction

Reaction with hydroxylamine produces the oxime (*a*), dehydration by acetic anhydride yields the acetylated nitrile (*b*), ammonolysis gives the cyanohydrin (*c*), loss of hydrogen cyanide leads to the next lower aldose (*d*). This then forms a diamide derivative (*e*), and is liberated by acidification with dilute hydrochloric acid (*f*):

(*a*) $HO{\cdot}CH_2{\cdot}HCOH{\cdot}CHO + H_2N{\cdot}OH \longrightarrow HO{\cdot}CH_2{\cdot}HCOH{\cdot}CH{:}N{\cdot}OH + H_2O$, then

(*b*) $HO{\cdot}CH_2{\cdot}HCOH{\cdot}CH{\cdot}N{\cdot}OH + 3Ac_2O \longrightarrow AcO{\cdot}CH_2{\cdot}HC(OAc){\cdot}C{:}N + 4Ac{\cdot}OH$, then

(*c*) $AcO{\cdot}CH_2{\cdot}HC(OAc){\cdot}C{:}N + 2NH_3/(alc.) \longrightarrow HO{\cdot}CH_2{\cdot}HCOH{\cdot}C{:}N + 2Ac{\cdot}NH_2$, then

(*d*) $HO{\cdot}CH_2{\cdot}HCOH{\cdot}C{:}N + Ag^+/(NH_3, alc.) \longrightarrow HO{\cdot}CH_2{\cdot}CHO + AgCN + H^+$, then

(*e*) $HO{\cdot}CH_2{\cdot}CHO + 2Ac{\cdot}NH_2/(alc.) \longrightarrow HO{\cdot}CH_2{\cdot}CH(HN{\cdot}Ac)_2 + H_2O$,[21] and

(*f*) $HO{\cdot}CH_2{\cdot}CH(NH{\cdot}Ac)_2 + H_2O/2HCl \longrightarrow HO{\cdot}CH_2{\cdot}CHO + 2Ac{\cdot}NH_3{}^{+-}Cl$

It is observed that this reaction is the reverse of the Kiliani synthesis. It was discovered by Wohl in 1893. Step *a* is the normal reaction of aldehydes and ketones with hydroxylamine. The acetic anhydride is used in *b* to effect dehydration, the acetylation of the hydroxyl groups being incidental. Steps *c*, *d*, and *e* are accomplished in a single operation by treating the acetylated nitrile with alcoholic ammoniacal silver nitrate. The ammonia effects ammonolysis of the ester linkages, and the silver nitrate accelerates the loss of hydrogen cyanide by the formation of insoluble silver cyanide. The reaction of the amide obtained by ammonolysis with the carbonyl group, as shown in equation *e*, is incidental, and this intermediate is decomposed in *f* by the addition of acid to liberate the aldopentoses. Reactions for effecting a similar result have been developed by Guebert, by Löb, by Neuberg, by Ruff, and by Weerman. These degradation reactions serve to confirm the relationships established by the use of the Kiliani synthesis and other reactions.

---

[21] This reaction is incidental as the acetamide formed by reaction *c* now reacts in turn with the free aldehyde group. Subsequent treatment with acid in equation *f* liberates the free sugar.

4. Controlled Oxidation of Polyhydroxy Alcohols

Controlled oxidation of polyhydroxy alcohols can be formulated as shown:

$$R_a \cdot CH_2OH + 2Br_2/H_2O/4C_6H_5CO_2Na, \text{aq.} \longrightarrow R_a \cdot CO_2H + 4NaBr + 4C_6H_5 \cdot CO_2H$$

The aldonic acid loses water to yield the aldonolactone which is then reduced with sodium amalgam in the presence of dilute sulfuric acid to yield the aldose sugar (see page 77). The bromite oxidation of the aldoses (that is, the simple sugars) was initiated by Kiliani and subsequently developed and greatly simplified by Isbell. A modification of the Isbell method may be used commercially for the production of gluconic acid, which is sold as its calcium salt as a metabolic source of calcium.

VIII. PHYSICAL PROPERTIES

The monosaccharides are sweet, water-soluble, crystalline compounds, but they are frequently crystallized with difficulty. The melting point of glycolaldehyde, glyceraldehyde, and dihydroxyacetone are, respectively, 95–97° C., 141–142°, and 68–75°, the range of the latter being ascribed to a tendency toward polymerization.

α-D^+-Glucose, m. p. 146°, has a specific rotation of +112.2, whereas the value for the β-isomer is +18.7 and that of the equilibrium mixture is +52.7. D^+-Galactose melts at 118–120° and D^+-mannose at 132°.

The di- and trisaccharides are crystalline and water soluble. Lactose loses its water of hydration at about 130°, undergoes caramel formation at 160–180°, and melts at about 200°. Maltose loses its water of hydration at about 100°. The osazone of lactose forms tufts of fine needles which are easily distinguished from the tufts of broader and blunter needles obtained from maltose. Sucrose melts at 186°, and on cooling solidifies to a white glassy mass, but upon prolonged heating above its melting point it solidifies on cooling to a brown product known as barley sugar. Caramel is produced by heating sucrose at 210°, at which temperature it loses water and acquires a brown coloration. Raffinose loses its water of hydration at 100–110° and melts at 119°.

Assigning a value of 100 to sucrose, the comparative sweetness of some of the common sugars is: D^--fructose, 173; D^+-glucose, 74; D^+-xylose, 40; maltose, 32; rhamnose, 32; D^+-galactose, 32; raffinose, 22; and lactose, 16.

The polysaccharides, in contrast with the simple sugars, are neither sweet, water soluble, nor crystalline. The dextrins have an insipid, mucilagenous taste. The so-called erythrodextrins[24] give a red coloration with the Wagner reagent (I_2/KI, aq.), whereas the achroödextrins give no coloration. Glycogen responds to this test to give a reddish-brown coloration. Air-dried starch is tasteless, contains about 13% water;

---

[24] These dextrins are ill defined, the achroödextrins representing a greater degree of degradation of the starch molecule than do the erythrodextrins.

but may be dried by heating at 100°. When starch is boiled with water, the bursting of the granules yields a colloidal solution which gives a blue coloration with the Wagner reagent. The coloration may result from an unstable complex as it disappears on heating and reappears on cooling. The properties of cellulose vary with its origin; it may be cellular, fibrous, or woody, and is of a collodial nature. Inulin is almost tasteless and gives a negative test with the Wagner reagent. The properties of some of the vegetable gums are described on page 819, *et. seq.*

IX. REACTIONS OF THE REDUCING SUGARS

1. Reduction

Electrolytic reduction yields polyalcohols such as sorbitol:

$$R_gCHO + 2H^* \text{ (electrolytic reduction)} \longrightarrow R_gCH_2OH^{25}$$

The reaction is used commercially for the production of polyhydroxy alcohols. D^+-Glucose, for example, gives sorbitol, which is used as a plasticizer. The reaction is applicable to either aldoses or ketoses. In the laboratory the reduction is effected by the use of sodium amalgam and water:

$$R_gCHO + 2Na/(Hg) + 2H_2O \longrightarrow R_g \cdot CH_2OH + 2NaOH$$

In the reduction of the ketoses, two isomeric alcohols are always produced as either of the two bonds of the carbonyl group may act as the hydrogen acceptor. The sugar acids (polyhydroxy acids) are reduced to the aldoses with sodium amalgam also, but in a slightly acidic medium, sulfuric acid being added dropwise as the reduction progresses (*cf.* reduction step in the Kiliani synthesis).

Catalytic hydrogenolysis yields a mixture of polyalcohols. D^+-Glucose, when subjected to catalytic hydrogenolysis, yields various cleavage products, the nature of the products depending on the catalyst, temperature, pressure, and time. Molasses under such treatment affords a potential source of useful hydroxy cleavage products.

Concentrated hydrogen iodide in the presence of red phosphorus yields hydrocarbons:

$$R_aCHO + 12HI/4P, \text{ heat} \longrightarrow CH_3(CH_2)_4CH_3 + 4PI_3 + 5H_2O$$

Iodoalkanes appear as intermediates, but the final products are the saturated hydrocarbons. The reaction is applicable to aldoses or ketoses and is used principally in the establishment of structural relationships. The aldonic acids (polyhydroxy acids) give the corresponding hydrocarbon acids. By the use of this reaction Kiliani obtained *n*-heptanoic acid from his hydrolytic product of glucose cyanohydrin, and thus established D^+-glucose as an aldose.

---

[25] Mannitol is a by-product of this reaction, which is operated commercially by the Atlas Powder Company.

2. Oxidation

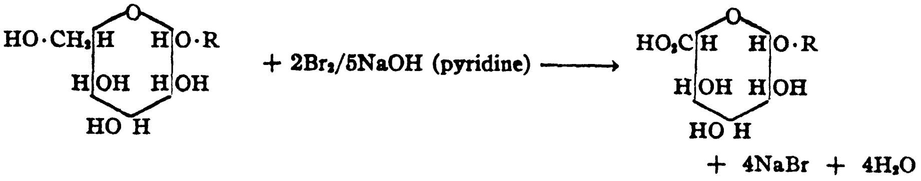
Oxidation of aldoses with bromine in mild alkali yields aldonic acids:

$$R_x\cdot CHO + Br_2/3Na_2CO_3/H_2O \longrightarrow R_x\cdot CO_2Na + 2NaBr + 3NaHCO_3$$

If sodium benzoate is used as the alkali, the liberated benzoic acid may be removed by filtration to give a filtrate which contains only the salt of the sugar acid and sodium bromide. The oxidation may be effected by the use of a number of solutions that contain a readily reducible ion. If the valence change of the ion is accompanied by some color change, a solution of the ion serves as a qualitative test for the presence of a reducing substance. The solutions most commonly used to indicate the reducing sugars are those of Benedict, of Fehling, and of Tollens. In the first two the essential change is represented by $Cu^{++} \rightarrow Cu^+$, which is precipitated as red to yellow hydrated cuprous oxide, and in the Tollens reagent the change is $Ag^+ \rightarrow Ag^0$, which is deposited as a silver mirror or appears as a dark colloidal suspension. The failure of the Schiff reagent to give positive results with the aldoses is ascribed to the fact that this change involves more than a simple oxidation-reduction reaction. The reagent appears to form a complex derivative with substances that are capable of removing sulfurous acid from the decolorized magenta, $H_2N\cdot C_6H_4\cdot C(SO_3H)(C_6H_4\cdot NH\cdot SO_3H)_2$. Ordinary aldehydes, organic amines, inorganic alkalies, certain hydrolyzable salts, and heat all effect such a change.

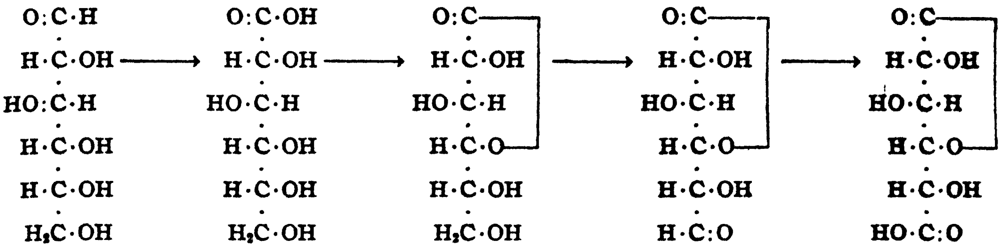
The oxidation of D^+-glucose is effected by fermentation or electrolytically on a commercial scale to produce gluconic acid which is converted in turn to calcium gluconate and marketed as a biological source of calcium, principally to supply calcium in a readily assimilable form to pregnant mammals.

Oxidation of glycosides with bromine in alkali and pyridine yields, by subsequent hydrolysis, uronic acids:



This equation represents the oxidation of a glucoside instead of a free sugar, but it is of interest because the subsequent acid hydrolysis of the oxidized derivatives results in the loss of the alkyl group and the formation of glucuronic acid ($HO_2C\cdot HCOH\cdot HCOH\cdot HOCH\cdot HCOH\cdot CHO$) which is employed as an intermediate in effecting the D^+-glucose to L^+-gulose inversion. D^+-Glucuronic acid, on progressive reduction, yields L^+-gulose. This oxidation illustrates, furthermore, the possibility of protecting a susceptible group and effecting the oxidation of a more resistant group.

Oxidation of aldohexoses with nitric acid yields mucic or saccharic acids or various products:



The reagent is nitric acid (sp. gr. 1.15) and the successive products are gluconic acid, gluconolactone, guluronolactone, and saccharolactone. The reaction affords an interesting example of progressive oxidation, the ease of oxidation decreasing as the reaction progresses. Characteristic of the hydroxy acids, the *lactone* is formed automatically in the presence of hydrogen ion. Mannosaccharic acid, which is the diacid derived from mannose, is reported as yielding a dilactone when subjected to heat. The corresponding diacid of galactose is mucic acid.

The application of chromic acid as oxidation agent opens theoretically the possibility for a great number of oxidized derivatives and cleavage products. According to patent claims, a prescribed set of experimental conditions will give appreciable quantities of a given derivative. Oxidative processes for the conversion of these materials to other commercially used chemicals such as tartaric acid and saccharic acid offer a definite challenge. With more severe experimental conditions, the ultimate products are carbon dioxide and water. Chromic acid is usually used to effect this later change in quantitative organic analysis for carbon by the wet method.[26]

3. Addition Reactions

The addition of hydrogen cyanide results in cyanohydrin derivatives:

$$R_x \cdot CHO + H \cdot CN/(\text{trace of } NH_3) \longrightarrow R_x \cdot CHOH \cdot CN$$

This is the first step in the Kiliani synthesis and reminds one of the corresponding reaction of the aldehydes and ketones with this reagent. The aldoses and ketoses do not, however, form additive products with the Grignard reagent or with sodium bisulfite. In the case of the Grignard reagent, the hydroxyl groups are acidic enough to effect its decomposition. It should be recalled, moreover, that only a limited number of the aldehydes and ketones form *well-defined* derivatives with sodium bisulfite.

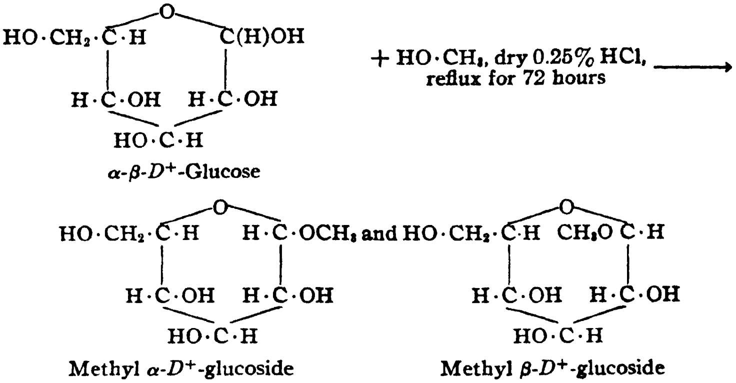
The addition of salts results in pseudo-addition products, an example of which is afforded by the formula $C_6H_{12}O_6 \cdot NaCl$. These unstable addition products are utilized in the commercial production of sucrose by treatment of the spent liquor with calcium or strontium oxide to yield insoluble derivatives which are filtered off and treated to recover both the sucrose and the metallic salts. Glucose reacts with ammonia to give glucose-ammonia and forms addition complexes with amino acids and with urea.

---

[26] C. B. Pollard and W. T. Forsee, *Ind. Eng. Chem., Anal. Ed.*, 7, 77 (1935).

4. Substitution

Replacement of the carbonylic H-atom by an alkyl group:



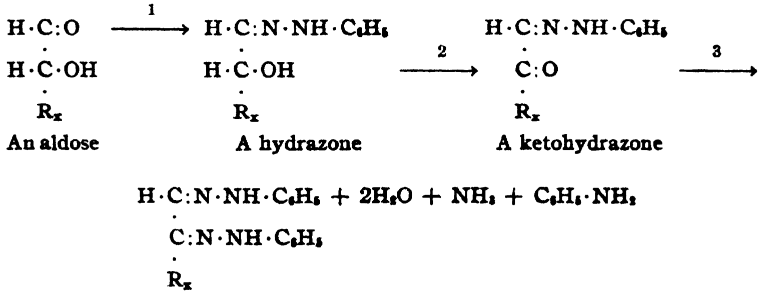
+ HO·CH₃, dry 0.25% HCl, reflux for 72 hours

α-β-D^+-Glucose

Methyl α-D^+-glucoside and Methyl β-D^+-glucoside

The α- and β-isomers are separated by fractional crystallization. The reaction is of particular interest because of the relative ease with which the carbonylic hydrogen atom is replaced. This is taken as additional evidence for the lactol structure of the carbohydrate units. The glucosidic linkage, moreover, is the one utilized by nature in building the polysaccharides from the monosaccharide units. As early as 1894, Emil Fischer discovered that the α-glucosidic linkage is hydrolyzed by *maltase* whereas the β-linkage is attacked by *emulsin*. This discovery has aided materially in establishing the type of linkage in the various di-, tri-, and polysaccharides. The biologically important phosphoric acid esters of the simple sugars belong to this group of derivatives in which the carbonylic hydrogen atom is replaced by an organic or inorganic residue.

Replacement of the carbonyl oxygen atom and the adjacent H- and OH-groups:



$$\begin{array}{l}H\cdot C{:}O\\ \cdot\\ H\cdot C\cdot OH\\ \cdot\\ R_x\end{array} \xrightarrow{1} \begin{array}{l}H\cdot C{:}N\cdot NH\cdot C_6H_5\\ \cdot\\ H\cdot C\cdot OH\\ \cdot\\ R_x\end{array} \xrightarrow{2} \begin{array}{l}H\cdot C{:}N\cdot NH\cdot C_6H_5\\ \cdot\\ C{:}O\\ \cdot\\ R_x\end{array} \xrightarrow{3}$$

An aldose — A hydrazone — A ketohydrazone

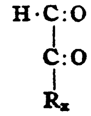
$$\begin{array}{l}H\cdot C{:}N\cdot NH\cdot C_6H_5\\ \cdot\\ C{:}N\cdot NH\cdot C_6H_5\\ \cdot\\ R_x\end{array} + 2H_2O + NH_3 + C_6H_5\cdot NH_2$$

An osazone

The reagent is phenylhydrazine ($H_2N\cdot NH\cdot C_6H_5$). The first step of the reaction is substitution with the loss of an equivalent of water and is perfectly analogous to the reactions of hydrazine and substituted hydrazines with aldehydes and ketones. The second step involves oxidation and reduction. The α-carbon group is oxidized to the carbonyl group, and the phenylhydrazine is reduced to ammonia and aniline. The third step is, once again, simple substitution, but only the mono- and di- derivatives are formed.

The osazones are crystalline derivatives that are useful in the characterization of the reducing sugars. This reaction, incidentally, aroused the interest of Emil Fischer in carbohydrate chemistry when he observed that glucosazone, fructosazone, and mannosazone are identical. This means, obviously enough, that the lower four carbon groupings in these compounds must be identical.

The osazones, when treated with concentrated hydrochloric acid, are hydrolyzed to the osones, a type formula of which is shown at the right. If the osones are treated with zinc dust and acetic acid, the terminal carbonyl group is reduced and a ketose sugar results. It is possible, therefore, to employ the phenylhydrazine and the osone reactions as a means of converting an aldose sugar to a ketose sugar. More specifically, D^+-glucose may be converted to D^--fructose by this series of reactions.



$$\begin{array}{c} H\cdot C{:}O \\ | \\ C{:}O \\ | \\ R_x \end{array}$$

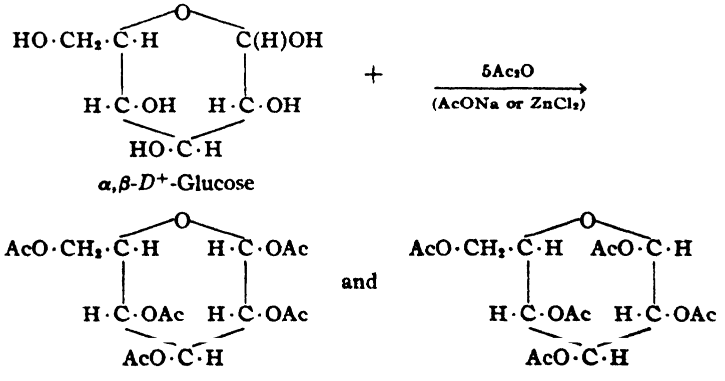
An osone

The first step in the Wohl degradation reaction is of this same general type:

$$R_x\cdot CHO + H_2N\cdot OH/(NH_3,\ alc.) \longrightarrow R_x\cdot CH{:}N\cdot OH + H_2O$$

The reaction has been considered in detail on p. 77 of this chapter.

Replacement of the H-atoms of the hydroxyl groups by acyl or alkyl:



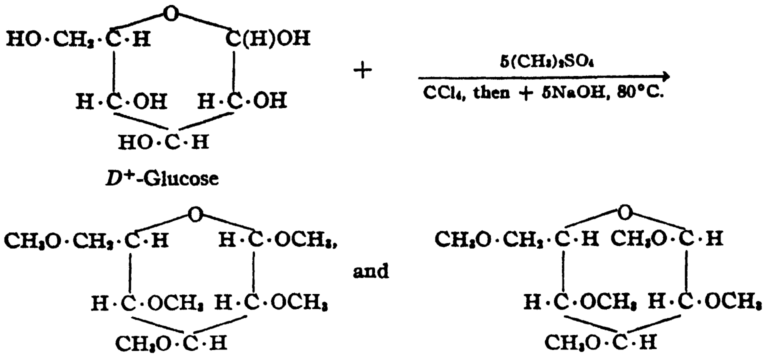
α,β-D^+-Glucose

Pentacetyl-α-D^+-glucose

Pentacetyl-β-D^+-glucose

These crystalline derivatives were first discovered by Franchimont in 1879. Little did he realize that his discovery was to form the basis for the cellulose acetate industry that was destined to usher in a new era—an age of cellophane, films, and artificial silks.

By varying the experimental conditions, the equilibrium between the pentaacetates may be shifted appreciably in the direction of either form. The isomeric forms may be separated by fractional crystallization.



D^+-Glucose

Methyl tetramethyl-α-D^+-glucoside

Methyl tetramethyl-β-D^+-glucoside

This reaction was developed by Purdie and Irvine in their studies of the configuration of the sugars. It, too, has found commercial application in the production of ethylcellulose which is used in the manufacture of transparent waterproof sheeting marketed as Ethocel.

Closely related to these reactions are those of the simple sugars with ethylene oxide, a reaction in which each hydroxyl group reacts with one equivalent of the oxide by direct addition with the regeneration of a new hydroxyl group that may react in turn with another equivalent of the oxide. This reaction, also, has definite commercial possibilities for the production of cellulose derivatives that will compete with the cellulose acetate industry.

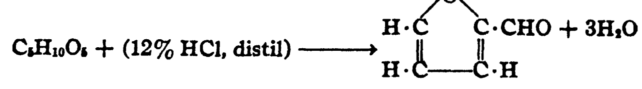
5. Fermentation

By fermentation of sugars under varying conditions, the following substances can be obtained: acetic acid, acetone, butanol, butanediols, citric acid, ethyl alcohol, fusel oils, gluconic acid, glycerol, lactic acid, tartaric acid, and other less important derivatives.

Since fermentation is considered elsewhere (see Vol. II, Chapter XII) in this treatise, further consideration will not be given to these derivatives at this time.

6. Dehydration

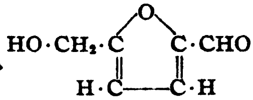
Pentoses dehydrated by 12% hydrochloric acid yield furfural:



The commercial source of the pentoses for this reaction is waste pentosans such as corncobs, cottonseed hulls, oat hulls, peanut shells, and straw. In commercial practice, oat hulls are so utilized and yield about 10 to 12% furfural. The furfural is used in the synthesis of Durite resins, in the solvent extraction process for the refining of lubricating oils, and as a synthetic intermediate.

Hexoses dehydrated by 12% hydrochloric acid yield hydroxymethylfurfural:

$C_6H_{12}O_6$ + (12% HCl, distil) $\longrightarrow$



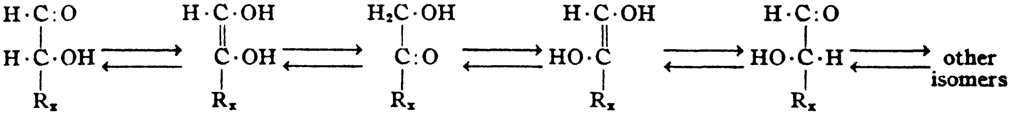
$+ 3H_2O$

7. Isomerization

With the help of mild alkali it is possible to convert one sugar isomer into another according to the following equation:

D^+-Glucose + mild alkali. aq. $\rightleftarrows$ D^--fructose $\rightleftarrows$ D^+-mannose $\rightleftarrows$ other isomers

The alkalies commonly used are the alkaline earths, sodium acetate, lead oxide, and guanidine. The equilibrium was discovered by Lobry de Bruyn and van Eckenstein and is responsible for the large variety of oxidized derivatives that are realizable in alkaline media. The isomerization is explained by the use of enolization:



$$\begin{array}{c}H\cdot C{:}O\\ |\\ H\cdot C\cdot OH\\ |\\ R_x\end{array} \rightleftarrows \begin{array}{c}H\cdot C\cdot OH\\ \|\\ C\cdot OH\\ |\\ R_x\end{array} \rightleftarrows \begin{array}{c}H_2C\cdot OH\\ |\\ C{:}O\\ |\\ R_x\end{array} \rightleftarrows \begin{array}{c}H\cdot C\cdot OH\\ \|\\ HO\cdot C\\ |\\ R_x\end{array} \rightleftarrows \begin{array}{c}H\cdot C{:}O\\ |\\ HO\cdot C\cdot H\\ |\\ R_x\end{array} \rightleftarrows \text{other isomers}$$

X. REACTIONS OF THE DI-, TRI-, AND POLYSACCHARIDES

1. Hydrolysis

The ultimate result of the hydrolysis of the higher saccharides are the monosaccharides which are their building units.

(*a*) Cellobiose + (cellobiase at 46° or acid) $\longrightarrow$ 2 D^+-glucose,
Lactose + (lactase or dilute acid) $\longrightarrow$ D^+-glucose + D^+-galactose,
Maltose + (maltase or dilute acid) $\longrightarrow$ 2 D^+-glucose,
Sucrose + (sucrase or dilute acid) $\longrightarrow$ D^+-glucose + D^--fructose.
(*b*) Cellulose + (cellulase at 67° or acid) $\longrightarrow$ *n* cellobiose,
Starch + (diastase or acid) $\longrightarrow$ *n* dextrins,
Dextrins + (diastase or dilute acid) $\longrightarrow$ *n* maltose,
Glycogen + (glycogenase or dilute acid) $\longrightarrow$ *n* D^+-glucose

The solution of cellulose by such reagents as hydrofluoric acid, phosphoric acid, and concentrated sulfuric acid is the result of hydrolysis.

2. Substitution

Substitution of hydrogen atoms of cellulose and starch results in a great number of technically important compounds.

(*a*) *Esterification*

Cellulose dissolves in an acetic acid-acetic anhydride-sulfuric acid mixture to give a clear solution. When this solution is poured into water, cellulose acetate is precipitated. If the precipitate is dried, dissolved in chloroform, and the chloroform allowed to evaporate, a cellulose acetate film is deposited. If the chloroform solution is forced through a spinneret, a thread is formed. The commercial application of this reaction gives us photographic films, filaments, and one type of artificial silk.

The lower esters of starch have been prepared and their properties compared with the corresponding derivatives of cellulose. The esters of starch, in general, are less satisfactory for the production of films than are the corresponding esters of cellulose.

(*b*) *Xanthation*

When cellulose is digested with 20% sodium hydroxide solution, pressed free of the alkali, treated with carbon disulfide, allowed to age, freed from excess carbon disulfide, and dissolved in 10% sodium hydroxide, a product is formed from which pure cellulose is precipitated in the form of a thread when the solution is forced through a spinneret into an acid bath. This series of reactions is the basis of the cellulose xanthate or viscose process, which accounts for about 80% of the artificial silk. In 1937, 1,725,000,000 lbs. of artificial silk were produced for world consumption.

Starch xanthates have been prepared, but their solubility in water makes isolation very difficult. The starch xanthates have been recommended for uses in mineral flotation and for other purposes.

(*c*) *Nitration*

Cellulose reacts with concentrated nitric acid in the presence of concentrated sulfuric acid to give cellulose nitrate. The control of the extent of nitration, within certain limits, determines the use of the nitrated product. Cellulose nitrate is used in artificial leather, bronzing liquids, collodion, dopes, explosives, lacquers, and plastics.

A limited amount of nitrated starch has been produced during the past few years for use as an explosive. Since the ordinary nitration procedure effects disintegration of the starch molecule more rapidly than it effects nitration, a technique was developed whereby the starch is sprayed with the nitrating agent.

(*d*) *Etherification*

Cellulose is converted into ethylcellulose by treatment with either ethyl chloride or ethyl sulfate in the presence of alkali. It is used in the production of transparent waterproof sheeting material under the trade name of Ethocel.

Ethers of starch have been prepared, but the methods of preparation are accompanied by appreciable amounts of disintegration of the starch molecule. The propyl and butyl ethers of starch, however, possess some of the film-forming properties which characterize the esters and ethers of cellulose.

Cellulose reacts with ethylene oxide in the presence of alkali to give direct addition products, the extent of the reaction being controlled by the amount of the reagent and the experimental conditions.

The ethylene oxide derivatives of starch have been prepared and studied, but to date (1942) they have not been commercialized.

3. Disintegration

When cellulose is treated with a specially prepared solution of ammoniacal cuprous chloride (Schweitzer reagent), it appears to dissolve. A colloidal solution is probably formed. If the solution is poured into acid, the cellulose is precipitated as individual fibers. This reaction is the basis of the cuprammonium process for the production of artificial silk.

4. Oxidation

Upon prolonged heating with concentrated nitric acid, cellulose yields carbon dioxide, oxalic acid, and water as the principal products. Concentrated sodium hydroxide has a similar effect. Starch, under controlled oxidation, gives appreciable yields of tartaric acid.

5. Fermentation

Hydrolysis normally precedes fermentation of both cellulose and starch. In certain cases, however, it seems likely that fermentation proceeds independently of hydrolysis. A member of the genus *Aerobacillus*, for example, effects the fermentation of starch to give 10.5 lbs. of 2,3-butanediol and 6.5 lbs. of ethyl alcohol per bushel of corn.

Selected Bibliography

Armstrong, E. F., and Armstrong, K. F., *The Carbohydrates.* Longmans, Green, London, 1934.
Bell, D. J., *Carbohydrate Biochemistry.* University Tutorial Press, London, 1940.
Degering, Ed. F., *Outline of the Chemistry of Carbohydrates.* John S. Swift & Co., Cincinnati, 1942.
Degering, Ed. F. *et. al., Outline of Organic Chemistry.* Barnes and Noble, New York, 1941.
Degering, Ed. F., "Notes on Sugar Chemistry", *J. Chem. Education*, **18**, 188 (1941).
Degering, Ed. F., "Starch Chemistry", *J. Chem. Education*, **18**, 45 (1941).
Gilman, H., *Organic Chemistry.* Wiley, New York, 1943.
Haworth, W. N., *The Constitution of Sugars.* Arnold, London, 1929.
Irvine, J. C., *The Carbohydrates.* Columbia Univ. Press, New York, 1927.
Meyer, K. H., *Natural and Synthetic High Polymers.* Interscience, New York, 1942.
Micheel, F., *Chemie der Zucker und Polysaccharide.* Akademische Verlagsgesellschaft, Leipzig, 1939.
Tollens, B., *Kurzes Handbuch der Kohlenhydrate*, Barth, Leipzig, 1935.

Chapter IV

LIPIDS

MANUEL M. BAIZER AND HUGO ZAHND
Brooklyn, New York

I. INTRODUCTION

The term *lipids* (*lipoids*[1]) was devised to provide a general name for several types of naturally occurring substances that differ chemically but are grouped together because of (*a*) similarities in solubility and (*b*) common capacity of being utilized by living organisms.[2]

The lipids include members of the following chemical species: sterols, aliphatic monocarboxylic acids—particularly those of relatively high molecular weight—and various esters and amides of these acids. The lipids are all soluble, to a greater or lesser extent, in the so-called fat solvents: hot ethanol, chloroform, carbon tetrachloride, petroleum ether, acetone, etc. Isolation of the lipids from their sources may be effected by either of two means: (*a*) extraction with one of the fat solvents, preferably boiling alcohol,[2] or (*b*) treatment with hot aqueous alkali followed by acidification with mineral acid. The former method has the advantage of removing the lipids as such; the latter method decomposes the acid derivatives but permits the study of the acids themselves.

II. CLASSIFICATION

Subdivision of the lipids into chemically homogeneous groups can be made on the basis of the types of products they yield on hydrolysis.

1. **Simple Lipids.**—On hydrolysis they yield only aliphatic alcohols and aliphatic monocarboxylic acids.

(*a*) *Fats and oils*—neutral esters of glycerol. The distinction between the two is based on their physical state at room temperature: the fats are solids, the oils liquids

(*b*) *Waxes*—neutral esters of monohydric aliphatic alcohols of relatively high molecular weight.

2. **Compound or Complex Lipids (Lipins[3]).**—On hydrolysis they yield aliphatic alcohols, aliphatic monocarboxylic acids, and additional products.

(*a*) *Phospholipids* (phosphatides).—The additional hydrolysis products are phosphoric acid and amines. The nature of the latter leads to further subclassification.

---

[1] British nomenclature.

[2] W. R. Bloor, *Chem. Revs.*, 2, 243 (1925).

[3] Frequent British usage.

(1) Lecithins. The amine is choline.
(2) Cephalins. The amine is colamine.
(3) Sphingomyelin. The amines are choline and sphingosine (an amino alcohol).

(*b*) *Cerebrosides* (glycolipids; galactolipids).—The additional hydrolysis product is a carbohydrate, generally galactose. Further subdivision can be made on the basis of the carboxylic acid produced.

(1) Kerasin. Yields lignoceric acid.
(2) Phrenasin. Yields cerebronic acid.
(3) Nervone. Yields nervonic acid.
(4) (Hydr)oxynervone. Yields (hydr)oxynervonic acid.

(*c*) *Sulfolipids and aminolipids.*—Comparatively little is known about these at the present time.[4]

A third major group, derived lipids, consisting of the fatty acids and the sterols, is often included in a classification scheme.

The "essential oils," responsible for the fragrance of many blossoms and fruits, are not lipids.

As a result of a long series of studies on the distribution in nature of the various fats and fatty acids, Hilditch[5] has evolved a novel and interesting scheme of classification. He has found that:

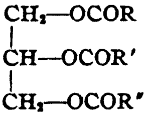
1. Plants and animals classed together biologically on the basis of their structural characteristics frequently elaborate similar kinds and proportions of fatty acids combined as glycerides.
2. The most intricate structures and most complex fatty acid mixtures are found in fats of the simplest forms of plant and animal life.[6]

III. SIMPLE LIPIDS

1. The Fats and Oils

(*a*) *The Glycerides*

Structure.—The fats and oils are glyceryl esters of aliphatic monocarboxylic acids (Table 6). The following generalized formula represents their structure:



$$\begin{array}{l} CH_2\text{—}OCOR \\ | \\ CH\text{—}OCOR' \\ | \\ CH_2\text{—}OCOR'' \end{array}$$

The hydrocarbon radicals represented by R, R′, and R″ may be all alike or all different; they may be saturated or they may contain double or triple bonds. If they are all alike, the ester is a *simple triglyceride;* if they differ, the ester is a *mixed triglyceride.* It is an empirical fact that the vast majority of natural fats and oils are of the mixed type. This leads Hilditch[5] to suggest that the fats (and oils) be defined as mixtures of mixed triglycerides.

---

[4] E. Chargaff, *J. Biol. Chem.*, **142,** 491 (1942). E. Klenk, *Z. physiol. Chem.*, **268,** 50 (1941). A. Petrun'kina, *Arkh. Biol. Nauk.*, **24,** 77 (1924); *cf. Chem. Abstracts*, **21,** 1484 (1927). M. Mitolo, *Boll. soc. ital. biol. sper.*, **8,** 69 (1933); *cf. Chem. Abstracts*, **27,** 3963 (1933).

[5] T. P. Hilditch, *The Chemical Constitution of Natural Fats.* Wiley, New York, 1940; *Ann. Rev. Biochem.*, **11,** 77 (1942).

[6] H. E. Longenecker, *Chem. Revs.*, **29,** 201 (1941).

TABLE 6
ACIDS DERIVED FROM THE SIMPLE LIPIDS

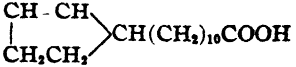
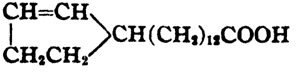
Acid	Formula	Occurrence	Melting pt., ° C.	Boiling pt., ° C.
		Saturated Acids		
Acetic	CH_3COOH	Oil of spindle tree	16.6	118.1
n-Butyric	$CH_3(CH_2)_2COOH$	Butter	−7.9	163.5
Isovaleric	$(CH_3)_2CHCH_2COOH$	Oils of dolphin and porpoise	−37.6	176.7
n-Caproic	$CH_3(CH_2)_4COOH$	Butter, coconut oil, palm-nut oil	−1.5	205
n-Caprylic	$CH_3(CH_2)_6COOH$	Butter, coconut oil, palm-nut oil	16	237.5
Capric	$CH_3(CH_2)_8COOH$	Cow and goat milk, coconut oil	31.5	268–270
Lauric	$CH_3(CH_2)_{10}COOH$	Milk, spermaceti, laurel oil, palm-kernel oil	44	225^{100}
Myristic	$CH_3(CH_2)_{12}COOH$	Nutmeg oil, milk and vegetable fats; lard and cod-liver oil	58	250.5^{100}
Palmitic	$CH_3(CH_2)_{14}COOH$	Most animal and vegetable fats; myrtle wax, bayberry tallow, beeswax	64	339–356 d.
Stearic	$CH_3(CH_2)_{16}COOH$	Most animal and vegetable fats	69.4	383
Arachidic	$CH_3(CH_2)_{18}COOH$	Peanut oil	76.3	328
Behenic	$CH_3(CH_2)_{20}COOH$	Oil of behen, butterfat	80.7 (84)	306^{60}
Lignoceric	$CH_3(CH_2)_{22}COOH$	Peanut oil, sphingomyelin, groundnut oil	81	. . .
Cerotic	Mixtures of homologs of $CH_3(CH_2)_{24}COOH$	Beeswax, carnauba wax, opium wax	87.7 (80–82)	d.
Melissic	$C_{30}H_{61}COOH$	Beeswax	91.9–92.1	. . .
		Acids with One Double Bond		
Tiglic	$CH_3CH{=}C(CH_3)COOH$	Croton oil	64	198.5
Hypogeic		Peanut and maize oils	33	236^{15}
Palmitoleic	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$	Seal oil		
Physetoleic		Sperm and seal oils		
Oleic	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	Most fats and oils	14	286^{10}
Rapic		Rape or colza oil		
Gadoleic	$CH_3(CH_2)_9CH{=}CH(CH_2)_7COOH$	Herring, sperm and cod-liver oils		
Erucic	$CH_3(CH_2)_7CH{=}CH(CH_2)_{11}COOH$	Rapeseed, mustard seed, cod-liver oils	33.5 (31–32)	281^{13}
Ricinoleic	$CH_3(CH_2)_5CHOHCH_2CH{=}CH(CH_2)_7COOH$	Castor oil	17	250^{15}
Hydnocarpic	 $\left.\begin{matrix}CH-CH\\ \vert\\ CH_2CH_2\end{matrix}\right>CH(CH_2)_{10}COOH$	Chaulmoogra oil		
Chaulmoogric	 $\left.\begin{matrix}CH{=}CH\\ \vert\\ CH_2CH_2\end{matrix}\right>CH(CH_2)_{12}COOH$	Chaulmoogra oil	68.5	$247\text{–}248^{20}$

TABLE 6 (*Continued*)
ACIDS DERIVED FROM THE SIMPLE LIPIDS

Acid	Formula	Occurrence	Melting pt., ° C.	Boiling pt. ° C.
Acids with Two or More Double Bonds				
Linoleic	$CH_3(CH_2)_4CH{=}CHCH_2$-$CH{=}CH(CH_2)_7COOH$	Cottonseed, linseed, poppyseed oils	−11	230[16]
Linolenic	$CH_3CH_2CH{=}CHCH_2CH{=}CH$-$CH_2CH{=}CH(CH_2)_7COOH$	Linseed, hemp, perilla oils	....	230–232[17]
Clupanodonic	$C_{22}H_{34}O_2$	Cod-liver, herring, sardine, whale oils	Less than −78	236[6]
Arachidonic	$C_{20}H_{32}O_2$	Liver tissue of pigs		
Acids with Triple Bonds				
Tariric	$CH_3(CH_2)_7C{\equiv}C(CH_2)_7COOH$	Picramnia seed fats		

It is obvious that mathematical combinations among the three R groups make possible a large number of mixed triglycerides. This number is even further increased by the fact that the terminal, or α-carbon, atoms differ from the central, or β-carbon, atom so that the following two esters are distinct structural isomers:

$$\begin{array}{l} CH_2OCOR \\ | \\ CHOCOR' \\ | \\ CH_2OCOR \end{array} \quad \text{and} \quad \begin{array}{l} CH_2OCOR \\ | \\ CHOCOR \\ | \\ CH_2OCOR' \end{array}$$

Finally, under certain circumstances, the central carbon atom may become asymmetric, thus leading to optical isomerism.

The identification of one particular triglyceride in a mixture of mixed triglycerides is an exceedingly difficult task.[7] Of major aid in an attempted solution of this problem has been fractional crystallization at low temperatures, molecular distillation,[8] and the study of the products of partial hydrogenation.[5, 9]

Hilditch and Meara[9] have proposed the following "rule of even distribution" and on the basis of this are working out a method of calculating from the fatty acid analysis of a natural fat the relative proportions of glycerides present. The method thus far has no theoretical basis, but in a number of instances cited calculated values are in excellent agreement with those found by the experimental methods mentioned above.

Rule of Even Distribution:

"1. If any individual acid (*e. g.*, oleic) forms one-third or more of the total molecules of fatty acids present in the fat, that acid will occur once (or more than once) in nearly all the triglyceride molecules.

"2. Any 'minor component' acid which does not form more than about 10% of the total molecules of fatty acids will contribute only one acyl group to such triglyceride molecules as may contain it."

---

[7] A symposium on the "Molecular Structure of Fats and Oils" together with numerous references to the original literature appears in *Chem. Revs.*, **29**, 199 (1941).

[8] N. D. Embree, *Chem. Revs.*, **29**, 317 (1941).

[9] T. P. Hilditch and M. L. Meara, *J. Soc. Chem. Ind.*, **61**, 117 (1942).

An illustration of the application of this principle is given by Hilditch and Meara.[9] Analysis of mowrah fat shows it to contain 24.1% palmitic, 19.3% stearic, 43.4% oleic, and 13.2% linoleic acid. (All percentages are mole percentages.)

The 56.6% "oleic" acid (oleic plus linoleic) is proportioned arithmetically between the two saturated acids, giving:

24.1% palmitic	19.3% stearic
31.4% "oleic"	25.2% "oleic"

Each group, divided up as a mixture of mono-"oleo"-disaturated glyceride and di-"oleo"-monosaturated glyceride, gives

38.4% palmito-di-"olein"	31.1% stearo-di-"olein"
17.1% "oleo"-dipalmitin	13.4% "oleo"-distearin

The calculated proportions of "oleo"-dipalmitin and "oleo"-distearin are then further resolved into 26.8% oleopalmitostearin and 3.7% "oleo"-dipalmitin.

The results as calculated above agree remarkably well with those obtained experimentally.

Nomenclature.—The nomenclature of glyceryl esters differs, in common usage, from that of other esters. Thus, glyceryl tristearate is called glyceryl tristearin, or, more simply, stearin. Named by this system, mixed triglycerides of oleic and stearic acids might be α-oleo-distearin, β-stearo-diolein, etc.

Physical Properties.—The fats and oils are soluble in the fat solvents previously mentioned and in each other. All but the glycerides of the acids of low molecular weight are insoluble in water.

The melting points of the fats and oils are higher than the solidification points. In the case of glycerides of saturated fatty acids, the melting point increases with increase in the molecular weight of the acids. Glycerides of unsaturated acids have a lower melting point than glycerides of saturated acids of the same carbon content. The oils, liquid at room temperature, contain a higher percentage of unsaturated acids than do the fats.

The fats all have a specific gravity of about 0.86; the variation from an average value is greater in the case of the oils (0.915–0.941).

Chemical Behavior.—Perhaps the most outstanding chemical property of the glycerides is their capacity of being hydrolyzed to glycerol and the constituent aliphatic acids. The hydrolysis may be accomplished by a variety of means: (*a*) by specific fat splitting, that is, lipolytic enzymes called lipases, (*b*) by superheated steam, (*c*) by superheated steam and a catalyst, (*d*) by alkali.

Method (*a*) is of particular interest in connection with digestion (see page 339). Methods (*b*) and (*c*) both produce glycerol and acids; the catalyst in Twitchell's process, a classic example of (*c*), is sulfuric acid plus an aromatic hydrocarbon or aromatic sulfonic acid. Method (*d*) produces alkali metal salts of the aliphatic acids, and since the alkali metal salts of the higher aliphatic acids are the soaps,[10] this process is called saponification.[11]

The *saponification number* of a fat or oil is the number of milligrams of potassium hydroxide needed to saponify completely 1 g. of the glyceride. Since this number varies inversely as the molecular weight of the glyceride, it serves as a measure of the mean molecular weight of the constituent acids.[12] Thus fats and oils containing low molecular weight fatty acids have high saponification numbers, for instance, butter and coconut oil.

Cholesterol and its esters, and certain vitamins that are associated with lipids in natural sources contribute largely to the "unsaponifiable matter"[13] found in greater or smaller amounts in most lipids.

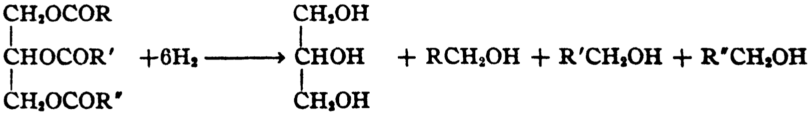
New variants of hydrolytic procedures, each striving for some particular advantage, are being devised constantly. At least one new method can be found in almost every issue of *Chemical Abstracts*. Very recently a 15-min. saponification at 220° in the presence of kerosene has been reported.[14]

Glycerides of unsaturated acids can add hydrogen at the multiple bonds. This process is carried out at elevated temperatures (180° C.), under pressure (10 atm.), and in the presence of a nickel catalyst.[15] Olein may be thus converted to stearin:

$$C_3H_5[OCO(CH_2)_7CH{=}CH(CH_2)_7CH_3]_3 + 3H_2 \rightarrow C_3H_5[OCO(CH_2)_{16}CH_3]_3$$

Inedible naturally occurring glycerides of unsaturated acids are converted by partial hydrogenation into the lard substitutes of commerce. (Complete hydrogenation would lead to the formation of brittle materials.) A synthetic lard may contain, *e. g.*, 20–25% of stearin (saturated), 60–65% of olein (unsaturated), and 5–10% of linolein (unsaturated).

The glycerides can undergo *hydrogenolysis:* cleavage by hydrogen into glycerol and the aliphatic alcohols corresponding to the constituent acids.



$$\begin{array}{l} CH_2OCOR \\ | \\ CHOCOR' \\ | \\ CH_2OCOR'' \end{array} + 6H_2 \longrightarrow \begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{array} + RCH_2OH + R'CH_2OH + R''CH_2OH$$

This may be achieved by the use of sodium and alcohols[16] or by hydrogen at 200° and 200 atmospheres pressure in the presence of a reduced basic copper chromite catalyst.[17]

---

[10] F. D. Snell, *J. Chem. Education*, **19**, 172 (1942).

[11] This term is often used loosely to include the other methods of ester hydrolysis.

[12] J. Lewkowitsch, *Chemical Technology and Analysis of Oils, Fats, and Waxes*, Macmillan, London, 1923. M. B. Jacobs, *Chemical Analysis of Foods and Food Products*, Van Nostrand, New York, 1938. Association of Official and Agricultural Chemists, *Official and Tentative Methods of Analysis*, 5th ed., Washington, D. C., 1940.

[13] H. Waelsch and W. M. Sperry, *J. Biol. Chem.*, **132**, 787 (1940).

[14] J. J. Jacobs, *Ind. Eng. Chem.*, **34**, 322 (1942).

[15] P. Sabatier, *Catalysis in Organic Chemistry*, Van Nostrand, New York, 1923. H. Adkins, *Reactions of Hydrogen with Organic Compounds*, Univ. of Wis. Press, Madison, Wis., 1937. C. Ellis, *Hydrogenation of Organic Substances*, Van Nostrand, New York, 1931.

[16] Bouveault and Blanc, *Bull. soc. chim.*, **31**, 1210 (1904).

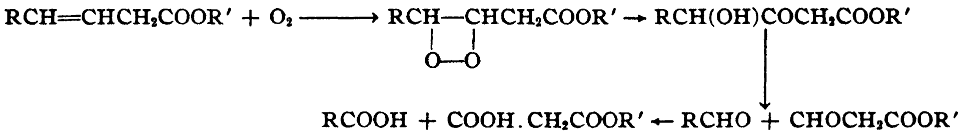
[17] H. Adkins and R. Connor, *J. Am. Chem. Soc.*, **53**, 1091 (1931). H. Adkins and K. Folkers, *ibid.*, **53**, 1095 (1931); U. S. Pat. 2,091,800 (Aug. 31, 1937); *cf. Chem. Abstracts*, **31**, 7444 (1937).

Unsaturated linkages are not affected. Hydrogenolysis of the glycerides makes possible the preparation of high molecular weight monohydric alcohols which are otherwise difficult to obtain.

Glycerides of unsaturated acids can add halogen at the multiple bonds. The halogen may be simple, such as iodine (Hübl), or of the mixed type, for example, iodine monochloride (Wijs) or iodine monobromide (Hanus). Thiocyanogen reacts similarly to the halogens.[18]

The *iodine number* of a fat or oil is the number of grams of iodine absorbed by 100 g. of the glyceride. It is a measure of the total amount of unsaturation in the constituent acids.[12]

Glycerides of unsaturated acids may be oxidized by addition of oxygen at the multiple bonds.[19] This leads in turn to the formation of peroxides, alcohols, aldehydes and ketones, and acids. Using a general ester formula to represent a glyceride, these steps may be thus summarized:



$$\begin{array}{l} RCH{=}CHCH_2COOR' + O_2 \longrightarrow \underset{|\quad\ \ |}{\overset{}{RCH{-}CH}}CH_2COOR' \rightarrow RCH(OH)COCH_2COOR' \\ \qquad\qquad\qquad\qquad\qquad\quad\ O{-}O \qquad\qquad\qquad\qquad\qquad\qquad \downarrow \\ \qquad\qquad\qquad\qquad RCOOH + COOH.CH_2COOR' \leftarrow RCHO + CHOCH_2COOR' \end{array}$$

Oils such as cottonseed, linseed, tung, which are glycerides of acids containing two or more double bonds (linoleic acid, linolenic acid), are called "drying oils" because they are converted by atmospheric oxygen into hard transparent films which can be used as varnishes and other protective coatings. Here the intermediate oxidation products have interacted to form resins.

The rancidity[20] which certain of the fats and oils suffer is due in part to oxidation by peroxidase present in certain molds and in part to enzymatic hydrolysis; evil-smelling aldehydes and free acids are produced. Rancidity of the fats and oils is hastened if they are exposed to light, or heated, or come into contact with metal.

Various substances, called inhibitols, serve as antioxidants and inhibit rancidity.[21] Natural antioxidants occur in wheat germ oil, cottonseed oil, and in other vegetables and vegetable oils. Synthetic antioxidants are phenolic compounds: catechol, hydroquinone, pyrogallol, etc. Recently the use of a number of hydroxamic acids as inhibitols has been patented.[22] The topic of antioxidants for fats and oils is discussed in Volume II, Chapter XIII.

Metabolic oxidation will be discussed later (page 105).

The *acid number* of a fat or oil is the number of milligrams of potassium hydroxide required to neutralize the uncombined aliphatic acid in 1 g. of sample. With certain limitations it is a measure of rancidity.

---

[18] H. P. Kaufmann and W. Oehring, *Ber.*, **59**, 187 (1926).

[19] E. Glimm, *Fette u. Seifen*, **46**, 348 (1939).

[20] C. H. Lea, *Rancidity in Edible Fats*, Chemical Pub. Co., New York, 1937. H. K. Dean, *Soap, Perfumery Cosmetics*, **14**, 307 (1941). H. Fiedler, *Fette u. Seifen*, **47**, 113 (1940). H. Süllman, *Helv. Chim. Acta*, **25**, 521 (1942).

[21] H. S. Olcott and H. A. Mattill, *J. Am. Chem. Soc.*, **58**, 1627, 2204 (1936).

[22] U. S. Pat. 2,279,973 (April 14, 1942); *cf. Chem. Abstracts*, **36**, 5372 (1942).

A summary of pertinent data on the simple lipids[23] is given in Table 7. These constants cover the commonly quoted range of maximum and minimum values. Actually, commercial fat and oil products are processed in such a manner that their constants fall within a narrow range. See page 581.

TABLE 7
COMMON FATS AND OILS
Mixtures of Mixed Triglycerides[a]

Common name	Solidification pt. (° C.)	Saponification no.	Iodine no.	Unsaponifiable matter
Beef tallow	31–38	196–200	35.4–42.3	.....
Butter fat	19–24.5	210–230	26–28	0.3–0.45
Castor oil	−17 to −18	175–183	84	0.6
Coconut oil	14–22	253.4–262	6.2–10	0.2
Cod-liver oil	−3	171–189	137–166	0.54–2.68
Cottonseed oil	12 to −13	194–196	103–111.3	1.1
Human fat	15	193.3–199	64	.....
Lard oil	4 to −2	193–198	62.5–79	1.1–1.6
Linseed oil	−19 to −27	188–195	175–202	0.4–1.2
Olive oil	−6	185–196	79–86	0.4–1.0
Palm oil	......	200–205	49.2–58.9	.....
Peanut oil	3	186–194	88–98	0.5–0.9
Porpoise body oil	−16	203.4	126.9	16–17
Seal oil	−13 to −18	188–203	122–141	.....
Sesame oil	−4 to −16	188–193	103–117	0.95–1.32
Soybean oil	−10 to −16	189–193.5	122–134	1.27–1.54
Tung oil	2–3	190–197	163–171	0.4–0.8
Wool fat	......	82–130	17–29	39–44

Single Triglycerides (for comparison)

Common name	Melting pt., ° C.	Saponification no., calcd.	Iodine no., calcd.
Stearin	54.5; 70.8	188	...
Olein	−17	190	86
Palmitin	65.1; 46	208	...
Oleo-distearin	......	190	28
Stearo-diolein	......	189	57
Dioleo-palmitin	......	195	59
Linolein	......	192	174

[a] Commercial fats and oils have narrow ranges. See page 581.

Synthesis.—Methods for the preparation of synthetic glycerides through the use of acetone glycerol were devised by Emil Fischer.[24] An interesting extension of these methods, involving tritylation, has been developed by Verkade[25] in a series of recent papers.

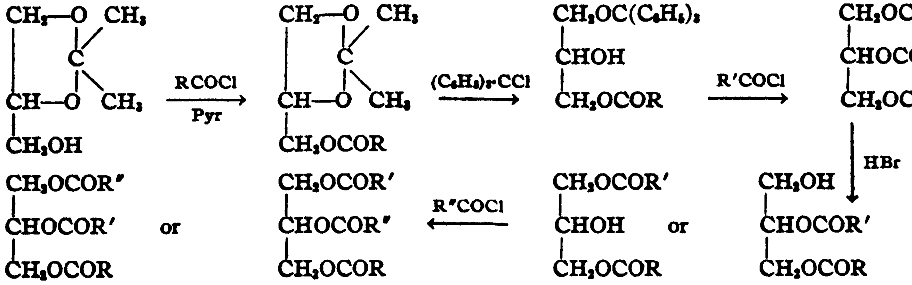
---

[23] C. L. Alsberg and A. E. Taylor, *The Fats and Oils*, Stanford Univ. Press, 1928. J. B. Leathes and H. S. Raper, *The Fats*, 2nd ed., Longmans, Green, New York, 1925.

[24] E. Fischer and E. Pfähler, *Ber.*, **53**, 1606 (1920).

[25] P. E. Verkade *et al.*, *Rec. trav. chim.*, **59**, 1123 (1940). P. E. Verkade and J. van der Lee, *ibid.*, **55**, 267 (1936). P. E. Verkade *et al.*, *Proc. Acad. Sci. Amsterdam*, **40**, 580 (1937). P. E. Verkade and J. van der Lee, *ibid.*, **37**, 812 (1934); *cf. Chem. Abstracts*, **29**, 2915 (1935). P. E. Verkade, *Fette u. Seifen*, **45**, 457 (1938).

Acetone glycerol is treated with an acyl chloride in the presence of pyridine or quinoline to yield an α-monoglyceride. This is then tritylated with triphenylchloromethane to an α-acyl-γ-tritylglyceride. Upon further acylation an α,β-diacyl-γ-tritylglyceride is formed which, on detritylation by hydrogen bromide, leaves one further position (γ- or sometimes β-) available for acylation.



(b) The Derived Acids

The acids[26] (Table 6) obtained by the hydrolysis of the simple lipids fall into three principal classes: (*a*) those that are saturated (fatty acids), (*b*) those that contain one double bond, (*c*) those that contain two or more double bonds. All are soluble in the fat solvents and in glycerides. The lower members of the first-mentioned series are water-soluble liquids; the intermediate members are liquids, and the higher members are of paraffin-like consistency. Those containing less than 10 carbon atoms are volatile with steam. Water solubility and acidity decrease with increasing molecular weight.

The number of milliliters of 0.1 *N* alkali required to neutralize the water-soluble and steam-volatile fatty acids from 5 g. of saponified and acidified fat is the *Reichert-Meissl number.*[12]

The lower members of series (*a*) can be shown by synthesis to contain straight chains of carbon atoms. One of the ways of proving this for the higher members is the degradative oxidation method of Krafft.[27]

$$(C_{17}H_{35}COO)_2Ba + (CH_3COO)_2Ba \xrightarrow{\Delta} 2BaCO_3 + C_{16}H_{33}CH_2COCH_3$$

$$C_{16}H_{33}CH_2COCH_3 \xrightarrow{\text{oxid.}} C_{15}H_{31}CH_2COOH + CH_3COOH, \text{ etc.}$$

The barium salt of stearic acid, *e. g.*, is mixed with barium acetate and dry distilled *in vacuo.* The ketone formed is converted by oxidation to the next lower acid. This procedure is continued until a low molecular weight acid has been reached whose structure has been independently elucidated.

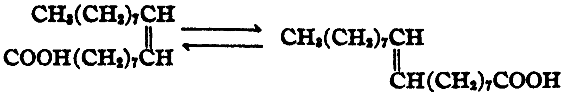
The lead salts of the unsaturated acids are much more soluble in ether than the corresponding salts of the saturated acids. This affords a method of separation.

Members of series (*b*), by virtue of their ethylenic linkage, exhibit geometric isomerism. Thus oleic acid may be transformed (by oxides of nitrogen) into its isomer, elaidic acid. (Oleic acid is the *cis*-isomer.) This elaidic transformation has been observed in other members of this class.

---

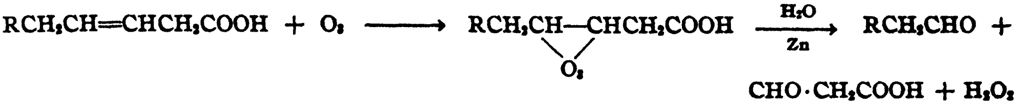
[26] Note that acids with an even number of carbon atoms predominate.

[27] Krafft, *Ber.*, 12, 1664 (1879).



$$\begin{matrix} CH_3(CH_2)_7CH \\ \| \\ COOH(CH_2)_7CH \end{matrix} \rightleftarrows \begin{matrix} CH_3(CH_2)_7CH \\ \| \\ CH(CH_2)_7COOH \end{matrix}$$

The position of the double bond in the molecule of an unsaturated acid may be determined by ozonolysis and hydrolysis.



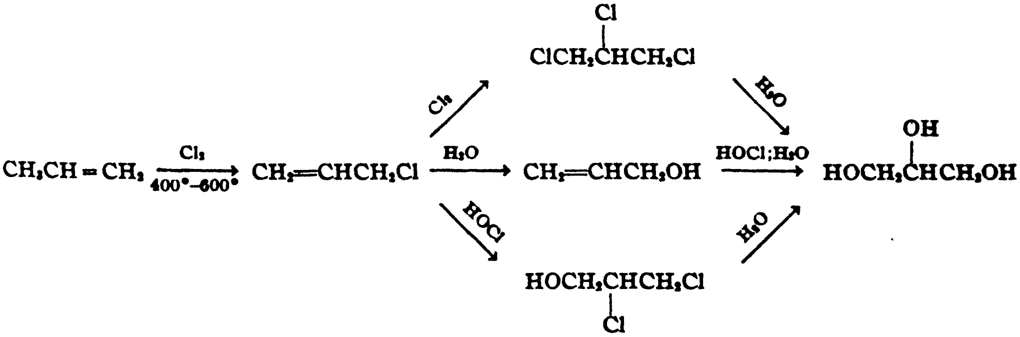
Identification of the aldehydes formed leads to the formulation of the structure of the original acid.

A few of the acids, of which the most important is ricinoleic acid, also contain hydroxyl groups and can therefore give reactions for alcohols. The number of milligrams of potassium hydroxide necessary to neutralize the acetic acid resulting from the hydrolysis of 1 g. of acetylated fat is the *acetyl number*.[28]

(*c*) *Glycerol*[29]

Glycerol (glycerin) is the most important of the trihydric alcohols. Its organic esters are, of course, the fats and oils. Chief in importance among its inorganic esters is glyceryl trinitrate (nitroglycerin). When the latter is absorbed in infusorial earth it forms dynamite.

Glycerol is obtained as a by-product of the soap industry. It is also made by the fermentation of glucose in the presence of sodium sulfite and synthetically from propene.[30]



---

[28] A. P. West *et al.*, *J. Biol. Chem.*, **104**, 627 (1934).

[29] J. W. Lawrie, *Glycerol and the Glycols*. Chemical Catalog Co., New York, 1928.

[30] E. C. Williams, *Ind. Eng. Chem.*, *News Ed.*, **16**, 630 (1938).

When glycerol is heated to an elevated temperature, especially in the presence of a dehydrating agent, it loses two molecules of water and forms acrolein (acraldehyde), $CH_2{=}CHCHO$. This substance is responsible for the acrid odor and lachrymatory vapors produced when fats are superheated.

2. Waxes

The lipid waxes are esters of aliphatic monohydric alcohols of high molecular weight and aliphatic carboxylic acids. They are widely distributed in the plant and animal kingdoms, their main function being apparently to serve as protective agents.[31] Waxes undergo hydrolysis with much more difficulty than do fats and oils, and advantage is taken of this fact in their separation. The more common derived alcohols are presented in Table 8. They can be obtained by hydrolysis or hydrogenolysis (see page 93).

TABLE 8

STRAIGHT-CHAIN ALCOHOLS DERIVED FROM WAXES

Common name	Formula	Occurrence	Melting pt., ° C.
Lauryl alcohol	$CH_3(CH_2)_{10}CH_2OH$	Sperm blubber oil	22.6
Myristyl alcohol	$CH_3(CH_2)_{12}CH_2OH$	Head oil of porpoise	37.6
Cetyl alcohol	$CH_3(CH_2)_{14}CH_2OH$	Sperm head oil, porpoise	49.3
Stearyl alcohol	$CH_3(CH_2)_{16}CH_2OH$	Sperm head, blubber oil	59
Oleyl alcohol	$CH_3(CH_2)_7CH{=}CH{-}(CH_2)_7{-}CH_2OH$	Sperm head, blubber oil	....
Linoleyl alcohol	$CH_3(CH_2)_4CH{=}CH{-}CH_2{-}CH{=}CH(CH_2)_7CH_2OH$	Sperm whale oil (?)	....
Ceryl alcohol	$CH_3(CH_2)_{24}CH_2OH$	Chinese wax	79.5–79.8
Melissyl alcohol	$C_{31}H_{63}OH$	Beeswax, carnauba wax	88

One particular class of alcohols derived from the waxes is of such importance as to have become a major branch of chemical investigation. These alcohols are the sterols, which are derivatives of cyclopentanoperhydrophenanthrene and chemically related to the bile acids and sex hormones.[32]

The sterols are, in general, stable crystalline compounds; they may occur free as well as combined with the higher fatty acids. They are isolated by ether or petroleum ether extraction of the unsaponifiable portion of a lipid fraction. The mixtures of sterols thus obtained may be difficult to separate.

The sterols may be subdivided into animal sterols (zoösterols), plant sterols (phytosterols), and sterols derived from yeast and fungi (mycosterols).

---

[31] W. R. Bloor, *Chem. Revs.*, 2, 243 (1925)

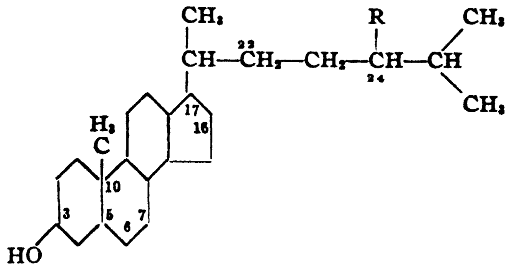
[32] L. F. Fieser, *Chemistry of Natural Products Related to Phenanthrene*, Reinhold, New York, 1937. H. Sobotka, *Chem. Revs.*, 15, 311 (1934). W. H. Strain, "The Steroids," in H. Gilman, *Organic Chemistry*, Vol. II, Wiley, New York, 2nd ed., 1943, p. 1341. R. E. Marker *et al.*, *J. Am. Chem. Soc.*, 64, 1653 (1942).

TABLE 9

SOME COMMON STEROLS

Name	Formula	Occurrence
Agnosterol	$C_{30}H_{48}O$	Wool fat
Cholesterol	$C_{27}H_{46}O$	All animal cells
Cinchol	$C_{29}H_{50}O$	Cinchona bark
Coprosterol	$C_{27}H_{48}O$	Feces
Dihydrocholesterol	$C_{27}H_{48}O$	With cholesterol
Ergosterol	$C_{28}H_{44}O$	Ergot, yeast
Fucosterol	$C_{29}H_{48}O$	Algae
Lanosterol	$C_{30}H_{50}O$	Wool fat
Ostreasterol	$C_{29}H_{48}O$	Oysters, gastropods
γ-Sitosterol	$C_{29}H_{50}O$	Fats of higher plants
Stigmasterol	$C_{29}H_{48}O$	Soybean
Zymosterol	$C_{27}H_{44}O$	Yeast

The following structural formula shows the general ring structure of all the sterols.



There are a number of points of asymmetry in the molecules leading to stereoisomerism; further, since the hydroxyl group on C_3 may be *cis-* or *trans-* to the methyl group on C_{10}, geometric isomerism is general among the sterols.

The sterols undergo certain characteristic color reactions which serve to distinguish unsaturated from saturated sterols and which are used in some cases for quantitative colorimetric determination.

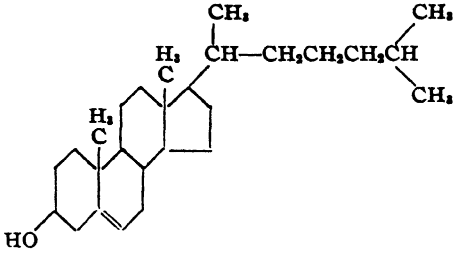
Liebermann or Liebermann-Burchard Reaction.—The solid sterol or a solution of the sterol in chloroform is treated with acetic anhydride and a few drops of concentrated sulfuric acid. The color changes from red to blue to green.

Salkowski Reaction.—A solution of the sterol in chloroform is shaken with concentrated sulfuric acid. A red color appears in the chloroform and a green fluorescence in the sulfuric acid.

Lifschutz Reaction.—To a solution of small quantities of the sterol and perbenzoic acid in glacial acetic acid is added concentrated sulfuric acid. The solution becomes violet-red, then blue, and finally green.

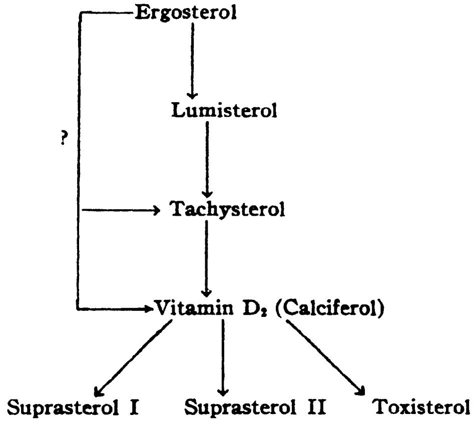
Rosenheim Reaction.—To a chloroform solution of the unknown are added a few drops of an aqueous solution of trichloroacetic acid. A red color develops which finally changes to a blue.

The most important of the zoösterols is cholesterol; it occurs in the lipid fraction of all organs, especially the brain and spinal cord, and in gallstones. It is probable that cholesterol may be the parent of the acids of bile and sex hormones. Further, it may serve as a protective agent for nervous tissue.



Cholesterol

The sitosterols[33] are important members of the phytosterols. Ergosterol, a mycosterol obtained from yeast and ergot, produces vitamin D_2 on irradiation (see page 247).



IV. COMPLEX LIPIDS

1. Phospholipids

The phospholipids[34] occur in every animal and vegetable cell, especially in brain, liver, heart, kidney, egg yolk, and bone marrow. They are miscible with both water and fats, and this is regarded as a significant fact in lipid metabolism (page 105). On complete hydrolysis they yield phosphoric acid, monocarboxylic acids, and: (*a*) glycerol plus choline (the lecithins); (*b*) glycerol plus colamine (the cephalins); (*c*) choline plus sphingosine (sphingomyelins).

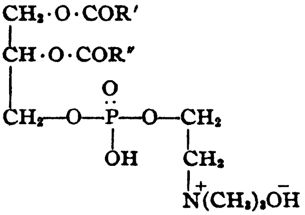
---

[33] E. S. Wallis and E. Fernholz, *J. Am. Chem. Soc.*, 58, 2446 (1936).

[34] H. Thierfelder and E. Klenk, *Die Cerebroside u. Phosphatide.* Springer, Berlin, 1930.

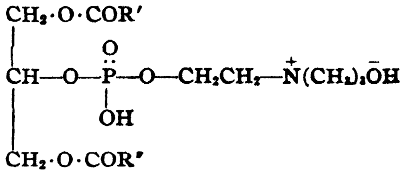
(*a*) *Lecithins*

The lecithins are of the following structures:



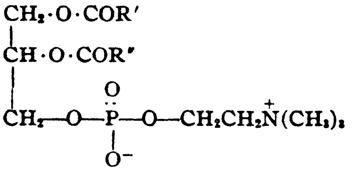
An α-lecithin

or



A β-lecithin

In addition to the isomerism shown above there are the possibilities of structural isomerism due to interchange of the R groups and of optical isomerism due to the asymmetry of the β-carbon atom. Internal neutralization may occur with the formation of a zwitter ion.[35]

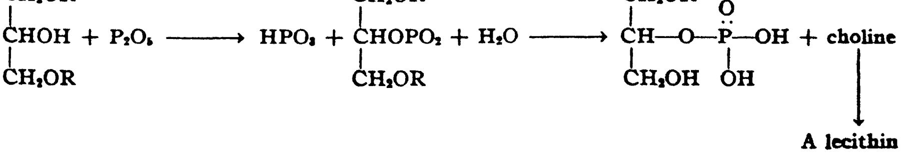


As in the case of the simple lipids, the R groups may be saturated or unsaturated, and the general rule is to find mixed types. Beef liver lecithin, for example, contains 40% saturated acids and 55% unsaturated acids.

Hydrolysis of the lecithins shown above yields first R′COOH, R″COOH, choline, and glycerophosphoric acid; on further hydrolysis the latter is decomposed into glycerol and phosphoric acid.

Lecithins may be separated from cephalins (with which they are associated naturally) by converting them to the cadmium chloride complex and then extracting with ethanol plus benzene or ethanol plus chloroform; the lecithin is thus obtained free from cephalin.[36]

Lecithins may be synthesized by treating a glycerol diester with phosphoric anhydride and then with choline:



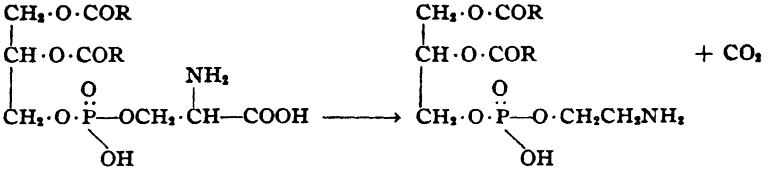
---

[35] H. Fischgold and E. Chain, *Proc. Roy. Soc. London*, **B177**, 239 (1935).

[36] V. A. Pertzoff and M. Aisner, *J. Urusvati Himalayan Research Inst.*, **2**, 133 (1932).

(b) *Cephalins*

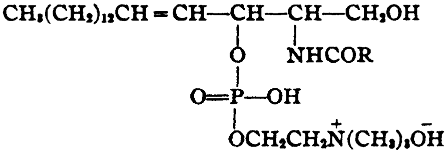
The cephalins[37] are distributed like the lecithins and can be separated from them by fractional crystallization from alcohol: the cephalins are less soluble. The structural formulas of the cephalins are similar to those of the lecithins except that colamine (β-aminoethanol) is substituted for choline. Recent work indicates that a hydroxyamino acid may take the place of part of the colamine.[38] This may be explained by postulating the relationship:



Both lecithins and cephalins are sensitive to air oxidation. On exposure, they turn yellow to brown in color.[39]

(c) *Sphingomyelins*

The sphingomyelins differ from the other phospholipids in yielding no glycerol on hydrolysis, and in being amides of acids (lignoceric, stearic, palmitic) instead of esters. They are white, crystalline, and relatively stable. On hydrolysis they yield choline, phosphoric acid, fatty acid, and the complex amino-alcohol sphingosine.[40,41] The following structure, coming after years of controversy regarding the number of carbon atoms in the chain and the position of substituents, seems most acceptable on the basis of recent work:



Many polemical papers have appeared since 1913 by Levene and by Klenk concerning the structure of sphingosine. The most recent formula proposed by Klenk and Diebold had given sphingosine the structure of 1,2-dihydroxy-3-aminoöctadecene-4.

"Sphingomyelin probably exists as a mixture of lignocerylsphingomyelin and lignocerylsphingomyelin fat acid ester."[42]

---

[37] H. MacLean and I. MacLean, *Lecithins and Allied Substances*. 2nd ed., **Longmans, Green,** London, 1937.

[38] G. Blix, *J. Biol. Chem.*, **139,** 471 (1941). J. Folch and H. A. Schneider, *ibid.*, **137,** 51 (1941).

[39] M. Bülow and I. H. Page, *Z. physiol. Chem.*, **205,** 25 (1932).

[40] E. Klenk, *Z. physiol. Chem.*, **185,** 169 (1929).

[41] E. Klenk and W. Diebold, *Z. physiol. Chem.*, **198,** 25 (1931). E. Klenk and F. Rennkamp, *ibid.*, **267,** 145 (1940). H. E. Carter *et al.*, *J. Biol. Chem.*, **142,** 449 (1942).

[42] S. J. Thannhauser and M. Reichel, *J. Biol. Chem.*, **135,** 1 (1940). M. Reichel and S. J. Thannhauser, *ibid.*, **135,** 15 (1940).

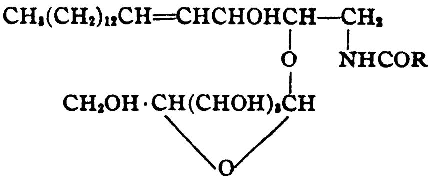
A review of the present status of phosphatide chemistry is given by Halden and Schilde[43] and by Kirk.[44]

Thannhauser and Reichel[42] are engaged in the synthesis of compounds in which both the hydroxyl groups of sphingosine are esterified but the amino group left free; these compounds show many typical properties of "fats."

2. Cerebrosides (Glycolipids; Galactolipids)

The cerebrosides occur chiefly in the brain and nerves. They are hydrolyzed by hot acid to galactose, the complex amine sphingosine, and fatty acids. The structural formula of a cerebroside shows it to be a glucoside. The carbohydrate portion of molecule undergoes typical reactions such as acetylation, and also contributes several asymmetric carbon atoms, so that the cerebrosides exhibit optical isomerism.

The cerebrosides[45, 46] are amides. In view of Carter's work,[41] which indicates that sphingosine is 1,3-dihydroxy-2-aminoöctadecene-4, Klenk and Härle's formula is in doubt:[47]



Name of cerebroside	Name of constituent acid	Formula of acid
Phrenasin	Cerebronic[48]	$C_{23}H_{47}CHOHCOOH$
Kerasin[47]	Lignoceric	$CH_3(CH_2)_{22}COOH$
Nervone[47, 49]	Nervonic	$CH_3(CH_2)_7CH{=}CH(CH_2)_{13}COOH$
(Hydr)oxynervone	(Hydr)oxynervonic	$CH_3(CH_2)_7CH{=}CH(CH_2)_{13}CHOHCOOH$

V. SOME BIOLOGICAL ASPECTS OF LIPID CHEMISTRY

1. The Role of Lipids in Nutrition

Of all the components of foodstuffs, the lipids provide the most concentrated source of energy. About 90% of their weight is combustible material; therefore a proper proportion of lipids in the diet reduces the bulk of necessary intake.[50–56] Although some lipids can be synthesized by the body from carbohydrates (page 107), it appears that at least two fatty acids[57] are nutritionally essential and must be supplied by the food.

---

[43] W. Halden and K. Schilde, *Fette u. Seifen*, **48**, 302 (1941).

[44] E. Kirk, *Ann. Rev. Biochem.*, **9**, 115 (1940).

[45] Recently E. Klenk[49b] announced the discovery of new lipids with high sugar content.

[46] E. Klenk, *Z. physiol. Chem.*, **145**, 244 (1925). P. A. Levene and C. J. West, *Proc. Soc. Exptl. Biol. Med.*, **14**, 93 (1917).

[47] E. Klenk and R. Härle, *Z. physiol. Chem.*, **189**, 243 (1930).

[48] F. A. Taylor and P. A. Levene, *J. Biol. Chem.*, **102**, 535 (1933). P. A. Levene and K. Heymann, *ibid.*, **102**, 1 (1933). P. A. Levene and P. S. Yang, *ibid.*, **102**, 541 (1933). E. Klenk and W. Diebold, *Z. physiol. Chem.*, **215**, 79 (1933). E. Klenk and L. Clarenz, *ibid.*, **257**, 268 (1939).

[49] (*a*) E. Klenk, *Z. physiol. Chem.*, **145**, 244 (1925); (*b*) E. Klenk and E. Schumann, *ibid.*, **267**, 128 (1941). E. Klenk, *ibid.*, **268**, 50 (1941).

The lipids that are not consumed to meet current energy needs may be stored in the body for use as fuel in the future. In this connection it should be noted that Schoenheimer,[56] using fats in which part of the hydrogen was substituted by deuterium, found that a large amount of fat is exchanged in the tissues even when an insufficient amount of it is administered in the diet.[58] It might be expected that rats fed on a diet insufficient to maintain body weight would burn all ingested fat for energy but actually about 50% of the tagged fatty acids turned up in the depot fat. As a result Schoenheimer concluded that there is a constant interchange between fed and stored fat even though the total amount of stored fat may remain constant or be decreased.

TABLE 10

PERCENTAGE OF LIPIDS IN SOME FOODS

Food	Lipid, %	Fuel value, cal./100 g.	Food	Lipid, %	Fuel value, cal./100 g.
Cottage cheese	1.0	112	Roast chicken	11.1	200
Swiss cheese	34.9	443	Duck	19	243
Scrambled eggs	18	210	Corn bread	4.7	266
Plain eggs	10.5	159	Rye bread	0.6	260
Clams	0.4	47.4	Corn flakes	0.4	383
Fresh halibut	5.2	125	Cocoa	28.9	511
Canned lobster	1.1	86.0	Raw apple	0.5	63.9
Fresh salmon	8.1	132	Bananas	0.6	101
Gelatin	0.1	376	Almonds	54.9	679
Cooked corned beef	26.2	308	Shelled peanuts	38.6	564
Beef liver	4.5	133	Dried Lima beans	1.5	358
Beefsteak, round	16.6	184	Cucumbers	0.2	17.6
Broiled lamb chops	29.9	367	Green olives	27.6	309
Frankfurters	18.6	258	Butter	85.0	795
Smoked bacon	64.8	646	Lard	100	930
Veal chops	10.0	174	Peanut oil	100	900
Buttermilk	0.5	36.4	Tomato soup	1.1	40.8
Skimmed milk	0.3	37.5			

Table 10 gives the percentage of lipids in several varieties of common foods together

---

[50] W. R. Bloor, *Chem. Revs.*, **2**, 243 (1925).

[51] H. C. Sherman, *The Chemistry of Food and Nutrition*. Macmillan, New York, 1937.

[52] A. J. Quick, *J. Biol. Chem.*, **77**, 581 (1928). W. E. Anderson and H. H. Williams, *Physiol. Revs.*, **17**, 335 (1937).

[53] I. Smedley-MacLean, *Ann. Rev. Biochem.*, **1**, 135 (1932); *ibid.*, **3**, 77 (1934).

[54] C. Chatfield and G. Adams, U. S. Dept. Agr., *Circ.* **146** (1931).

[55] A. L. Winton and K. B. Winton, *The Structure and Composition of Foods*, 3 vols., Wiley, New York, 1937.

[56] Atwater and Bryant, Office Expt. Sta., U. S. Dept. Agr., *Bull.* **28**.

[57] Linoleic and linolenic. Arachidonic acid may be essential or may substitute for one of these two.

[58] R. Schoenheimer, *Science*, **87**, 222 (1938). *The Dynamic State of Body Constituents*, Harvard Univ. Press, 1942, p. 3.

with the fuel value of that food. In general, vegetables and fruits have small lipid content, whereas meat and dairy products have high lipid content. The fuel value becomes of greater significance when the lipid percentages are taken in conjunction with the protein and carbohydrate percentages.[59]

The lipids are further important as sources of certain vitamins which are associated with them naturally.

2. Lipid Metabolism

The fats are hydrolyzed—but not completely[60]—in the villi of the small intestine by the lipolytic enzyme lipase into glycerol and acids. The formerly accepted view that the free acids were then carried along in the form of soaps is, in view of the *p*H of the medium, at least questionable. In any case, the acids are resynthesized in the intestinal wall into fats—not necessarily identical with the original ones—taken up mainly by the lymph vessels, and poured into the blood. The resynthesized fat may now undergo combustion or be stored.

Most investigators agree that the glycerol is oxidized to glyceric aldehyde or some similar 3-carbon-atom molecule and is ultimately, like the carbohydrates, converted to carbon dioxide and water. The ultimate fate of the acids is likewise carbon dioxide and water, but the steps by which these final combustion products are reached cannot be said to be clearly established. Several theories have been proposed and they each possess merit and experimental support. But so long as the fact remains that only a few of the intermediate products of normal metabolism have been isolated or otherwise identified, portions, at least, of these theories must be regarded as plausible conjecture.

There is difference of opinion, first of all, concerning the initial reactions that render the acids unstable and susceptible to oxidative destruction. One view is that phosphorylation engenders this instability; another proposes that the acids are first desaturated in the liver. Multiple linkages in the molecule would presumably provide points of attack, but this point too is in doubt.

Perlman *et al.*,[61] fed to fasted rats disodium phosphate in which the phosphorus was the P^{32} isotope. Their results indicate that the liver, small intestine, and possibly the kidney actually participate in the synthesis of phospholipids, although the possibility exists that these organs merely concentrate the phospholipids.

Hunter,[62] using a similar experimental technique, found that radioactive phosphorus is incorporated into sphingomyelin to the greatest extent in the liver, lungs, and intestinal mucosa. The total phospholipid fraction in the liver was many times more reactive than the sphingomyelin.

Perlman and Chaikoff[63] found that choline accelerates the synthesis and transfer of phospholipid by the liver of the fat-fed rat.

---

[59] C. D. Hodgman and H. N. Holmes (Editors), *Handbook of Chemistry and Physics*. 26th ed., Chemical Rubber Pub. Co., Cleveland, Ohio, 1942, p. 1435.

[60] Fraser, *Analyst*, **63**, 308 (1938).

[61] I. Perlman *et al.*, *J. Biol. Chem.*, **122**, 169 (1937).

[62] F. E. Hunter, *Proc. Soc. Exptl. Biol. Med.*, **46**, 284 (1941); *cf. Chem. Abstracts*, **35**, 2977 (1941). B. Cavanagh and H. S. Raper, *Biochem. J.*, **33**, 17 (1939).

[63] I. Perlman and I. L. Chaikoff, *J. Biol. Chem.*, **127**, 211 (1939).

The following are the principal mechanisms that have been proposed for the metabolism of the fatty acids:

(a) *The Theory of β-Oxidation (Knoop)*

The long carbon chains of fatty acids are oxidized two carbons at a time with the formation of acetic acid and one ketone molecule per molecule of fatty acid. The oxidation is usually represented by dehydrogenation followed by hydrolysis, although the enzyme systems involved have not yet been isolated. An analogous system postulates that the acids are converted by organic peroxides to β-hydroxy acids and then to the corresponding β-keto acids. The molecules are now cleaved at the β-positions and give rise to acids containing 2 carbon atoms less than the original ones. This sequence is continued until acetoacetic acid is produced. The assumption that "fats burn in the flame of carbohydrates" is frequently associated with the β-oxidation hypothesis, but is not necessarily a part of it.

In the absence of carbohydrates, the final stages of the oxidation do not take place and the end products are the so-called acetone bodies such as acetoacetic acid. Formation of these compounds is called ketogenesis or ketosis; their secretion in the urine is ketonuria or acetonuria. The condition is prevalent in diabetes, starvation, and phosphorus poisoning.[64]

$$\begin{tikzcd}
\mathrm{RCH_2CH_2COOH} + (\mathrm{O}) \arrow[r] & \mathrm{RCH(OH)CH_2COOH} + (\mathrm{O}) \arrow[r] & \mathrm{RCOCH_2COOH} \arrow[dl, "-\mathrm{CO_2}"'] \\
\mathrm{RCOOH} & (\mathrm{O}) + \mathrm{RCOCH_3} \arrow[l] &
\end{tikzcd}$$

or, if initial desaturation is postulated,

$$\mathrm{RCH_2CH_2COOH} \longrightarrow \mathrm{RCH{=}CHCOOH} + \mathrm{H_2O} \longrightarrow \mathrm{RCH(OH)CH_2COOH}, \text{ etc.}$$

Dakin has shown that butyric acid can be oxidized *in vitro* by 3% hydrogen peroxide first to β-hydroxybutyric acid and then to acetoacetic acid.

The evidence for β-oxidation rests primarily on three lines of experimental evidence: (*1*) Direct evidence of β-oxidation when phenyl substituted fatty acids of 1 to 4 carbon atoms were fed to dogs. Evidence of δ-oxidation when phenylvaleric acid was fed to dogs. This was interpreted by Dakin as successive β-oxidation. (*2*) Perfusion of livers of diabetic dogs with fatty acids led to the accumulation of ketone bodies. This was interpreted as being consistent with Knoop's hypothesis. (*3*) The recent evidence by Schoenheimer and coworkers that stearic acid ⇄ palmitic acid and oleic acid ⇄ palmitic acid in the animal body.

On the negative side is the fact that neither the lower fatty acids (10 to 4 carbon atoms) nor acetic acid have been isolated from the animal body in amounts predicted by the Knoop hypothesis.

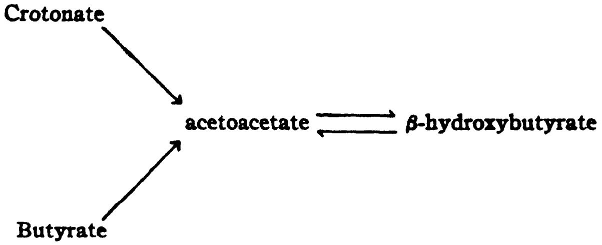
---

[64] S. Soskin and R. Levine, *Arch. Internal Med.*, **68**, 674 (1941).

(*b*) *The Theory of Multiple Alternate Oxidation*

The molecule of acid is considered to be oxidized at the β-position and then, receding from the carboxyl group, at every alternate carbon atom before oxidation to acetoacetic acid takes place. That is, according to this hypothesis, a fatty acid is simultaneously oxidized along the entire chain at alternate carbon atoms. Thus the fatty acid, such as palmitic acid, is totally disrupted and yields four molecules of ketones per molecule of fatty acid, in contradistinction to the Knoop hypothesis which postulates that only one molecule of ketone is formed per molecule of fatty acid.

Jowett and Quastel are of the opinion that since "the yield of ketone bodies from butyric acid is much less than from the higher acids, it is therefore very unlikely that the higher acids yield ketone bodies exclusively by a mechanism by which they first break down into butyric acid."[65] They favor the scheme



(*c*) *The Theory of ω-Oxidation*[66]

The terminal methyl group (ω- with respect to the carboxyl group) is oxidized to carboxyl. The dicarboxylic acid thus produced is then biterminally oxidized at the β-positions. This theory suggested itself to Verkade as a result of the accidental discovery of dioic-acid acidosis and diaceturia in man after administration of triundecylin.

Further details concerning the metabolism of fatty acids and other lipids may be found in the given literature.[67–71]

3. Biological Synthesis of Lipids

There are strong indications that plants are able to convert part of their carbohydrates into fat. In the case of animals, the evidence for such a synthesis in incontrovertible.

---

[65] M. Jowett and J. H. Quastel, *Biochem. J.*, **29**, 2143 (1935).

[66] P. E. Verkade *et al.*, *Z. physiol. Chem.*, **250**, 47 (1939). P. E. Verkade, *Chemistry & Industry*, **1938**, p. 704.

[67] W. M. Sperry *et al.*, *J. Biol. Chem.*, **135**, 281 (1940).

[68] K. Bernhard, *Helv. Chim. Acta*, **24**, 1412 (1941).

[69] H. B. Bull, *The Biochemistry of the Lipids*. Wiley, New York, 1937; W. R. Bloor, *Biochemistry of the Fatty Acids and Their Compounds, the Lipids*. Reinhold, New York, 1943.

[70] H. C. Eckstein, *Ann. Rev. Biochem.*, **10**, 181 (1941).

[71] E. Chargaff, *Ann. Rev. Biochem.*, **11**, 235 (1942).

Sherman[72] cites the following types of evidence:

1. Milch cows fed on a fat-free diet gave about twice as much milk fat as could be accounted for from the fat and protein in their food.
2. Animals fattened on carbohydrate food much more than could be accounted for by the fat and protein given in their diet.
3. Evidence obtained from the respiration quotient.
4. Evidence obtained from an investigation of the total carbon stored in the body by animals being fattened on carbohydrate food.

Here again, however, the question of the mechanism whereby carbohydrates are converted into fat has given rise to much speculation. Some of the theories that have been proposed are:

1. Lactic acid, from the metabolism of carbohydrates, can give rise to acetaldehyde. The latter can form aldol, which, on disproportionation, would give rise to butyric acid, or, in a series of aldol condensations with itself or with aldol followed by internal oxidation-reductions, would give rise to higher aliphatic acids.

$$CH_3CHO + HCH_2CHO \longrightarrow \underset{\text{Aldol}}{CH_3CH(OH)CH_2CHO} \longrightarrow \underset{\text{Butyric acid}}{CH_3CH_2CH_2COOH}$$

Kuhn *et al.*,[73] condensed crotonaldehyde in the presence of piperidine salts and obtained $CH_3(CH{=}CH)_7CHO$. The latter, when condensed with malonic acid and hydrogenated, formed stearic acid. Kuhn considers this reaction of significance for biological synthesis because it derives from room temperature catalytic condensations of acetaldehyde molecules.

2. Pyruvic acid, likewise formed in carbohydrate metabolism, may condense with aldehydes. The product splits out carbon dioxide and yields an acid containing 2 more carbon atoms than the original aldehyde.

$$CH_3CHO + HCH_2COCOOH \longrightarrow CH_3CH(OH)CH_2COCOOH \xrightarrow{-CO_2} CH_3CH(OH)CH_2CHO$$

3. Hexoses[74] may break down into C_3 units which then condense to fatty compounds containing 6 and 9 carbon atoms; the latter two then condense with themselves to form C_{12} and C_{18} compounds. (In this connection it is known that under certain abnormal conditions fatty acids with an uneven number of carbon atoms are degraded to propionic acid and this goes through lactic acid to glucose.)

The problem of the biological synthesis of lipids is a complex one and much remains to be done before some of the tentative hypotheses can be ruled out or more firmly established. The discovery of the fatty aldehyde palmital by Feulgen and Bersin[75] and its esterification with glycerophosphate, as well as the work of Schoenheimer, Ratner, and Rittenberg[76] using deuterium or radioactive carbon as tracers, are important contributions.

---

[72] H. C. Sherman, *The Chemistry of Food and Nutrition.* Macmillan, New York, 1937.

[73] R. Kuhn *et al.*, *Z. physiol. Chem.*, **248**, IV (1937).

[74] E. F. Armstrong and J. Allan, *J. Soc. Chem. Ind.*, **43**, 216T (1924). I. Smedley-MacLean, *Ergeb. Enzymforsch.*, **5**, 285 (1936).

[75] R. Feulgen and T. Bersin, *Z. physiol. Chem.*, **260**, 217 (1939).

It is known[77] that proteins may give rise to carbohydrates; as a corollary proteins may be involved in the biosynthesis of lipids. The synthesis by the body of lipids of complex structure, such as cholesterol,[78] has also been established, but even less is known about their mode of formation.

---

[76] R. Schoenheimer, S. Ratner, and D. Rittenberg, *J. Biol. Chem.*, **127,** 333 (1939); *ibid.*, **130,** 703 (1939).

[77] H. H. Mitchell and T. S. Hamilton, *The Biochemistry of the Amino Acids.* Chemical Catalog Co., New York, 1929.

[78] R. Schoenheimer and F. Breusch, *J. Biol. Chem.*, **103,** 439 (1933).

Selected Bibliography

Bloor, W. R., *Biochemistry of the Fatty Acids and Their Compounds, the Lipids.* Reinhold, New York, 1943.

Gilman, H., *Organic Chemistry.* Wiley, New York, 1943.

Hilditch, T. P., *The Chemical Constitution of Natural Fats.* Wiley, New York, 1940.

Hilditch, T. P., *The Industrial Chemistry of the Fats and Waxes.* Van Nostrand, New York, 1941.

Jacobs, M. B., *Chemical Analysis of Foods and Food Products.* Van Nostrand, New York, 1938.

Jamieson, G. S., *Vegetable Fats and Oils.* Reinhold, New York, 1943.

Lea, C. H., *Rancidity in Edible Fats.* Chemical Pub. Co., New York, 1937.

Lewkowitsch, J., *Chemical Technology and Analysis of Oils, Fats and Waxes.* Macmillan, New York, 1923.

MacLean, H., and MacLean, I., *Lecithins and Allied Substances.* Longmans, Green, London, 1939.

Sherman, H. C., *Chemistry of Food and Nutrition.* MacMillan, New York, 1937.

Chapter V

SOME ASPECTS OF THE CHEMISTRY OF AMINO ACIDS AND PROTEINS

HUGO ZAHND AND MANUEL M. BAIZER

Brooklyn, New York

I. INTRODUCTION

The term *protein* is used for a group of complex nitrogenous organic substances present in all protoplasm of vegetable and animal cells. The function which proteins play in the very life processes of living matter cannot be overemphasized. Whereas carbohydrates and fats serve mainly as energy producers, the proteins are mainly used for the repair of worn and the building up of new tissue. Plants, yeasts, and bacteria build their own proteins from inorganic substances, but the higher animals no longer possess this power. Animals and man, therefore, depend on the proteins in the diet for the materials out of which they construct the complex nitrogenous substances characteristic of the species to which they belong. Active tissues, such as nerve, muscle, skin, hair, and nail, consist largely of protein material. In general, proteins, unlike carbohydrates and fats, are not stored in the plant and animal organism; that is to say, the protein in excess of metabolic requirements is not stored in the form of a reserve supply, except when this food is required for formation of the tissues of rapidly developing young plants or animals. In this case it is found in such sources as the seeds of the plant, the white of the egg, or the milk of the mother.

The progress in the field of protein chemistry has been slow but steady. New chemical and physicochemical methods and tools had to be developed in order to study the very complex structure and physicochemical properties of these substances. The subject is still a matter of most active research and its problems challenge the skill and ingenuity of the most gifted investigators. Substantial progress, particularly in the field of proteins as related to nutrition, has been made; yet, many fascinating but difficult problems await solution. The reader interested in the historical development of this branch of chemistry will find the given literature instructive reading.[1–6]

II. CLASSIFICATION OF PROTEINS

Proteins, as will be shown later in this treatment, can be decomposed by chemical means into 20 or more "building blocks," the amino acids. It has further been found that proteins of different tissue are not identical in their chemical and physical properties, nor are the proteins of the same tissue of different species the very same. These proteins, of which there are a very large number, have been shown to possess most complex structures. Generally speaking, the products of hydrolysis of different proteins may vary in qualitative composition and always differ in quantitative composition. In no case are we certain of the exact mode of linkage between the building blocks which go into the structural make-up of the protein molecule. To arrive at a solution of the structure problem, almost insurmountable difficulties have to be overcome. The nature of the problem becomes clearer when we realize that the lowest molecular weight of a real protein is about 17,000,[6a] while hemocyanin (from helix blood) is assigned a value of 6,700,000. Incomplete knowledge of chemical structure prevents a *chemical* classification, and proteins are therefore grouped according to their sources, differences in solubility, and the chemical components found in the product of hydrolysis. The classification which follows is based upon the recommendations made by a joint committee of the American Physiological Society and the American Society of Biological Chemists which met in 1907.

1. Simple Proteins

Simple proteins are substances which upon hydrolysis yield amino acids or their derivatives. This definition is not completely inclusive, since carbohydrate substances have recently been reported in the hydrolyzates of "pure" proteins.

1. *Albumins* are soluble in water, coagulated by heat, and are often wanting in glycine. Examples: egg albumin, serum albumin (from blood), lactalbumin (from milk), leucosin (from wheat), legumelin (from peas).
2. *Globulins* are insoluble in pure water but soluble in strong acids, alkalies, and neutral salt solutions. These proteins usually contain glycine. Examples: myosin (from muscle), serum globulin (from blood), edestin (from hempseed and other seeds), ovoglobulin (from yolk), phaseolin (from beans), legumin (from peas), amandin (from almonds), arachin (from peanuts), excelsin (from Brazil nuts).
3. *Glutelins* are soluble in dilute acids and alkalies but insoluble in neutral solvents. Example: glutenin (from wheat).

---

[1] C. L. A. Schmidt, *The Chemistry of the Amino Acids and Proteins*. Thomas, Springfield, 1938.

[2] H. B. Vickery and C. L. A. Schmidt, *Chem. Revs.*, 9, 169 (1931).

[3] H. H. Mitchell and T. S. Hamilton, *The Biochemistry of the Amino Acids*. Chemical Catalog Co., New York, 1929.

[4] H. B. Vickery and T. B. Osborne, *Physiol. Revs.*, 8, 393 (1928).

[5] J. Loeb, *Proteins and the Theory of Colloidal Behavior*. McGraw-Hill, New York, 1924.

[6] R. H. A. Plimmer, *The Chemical Constitution of the Proteins*. Longmans, Green, London, 1917

[6a] Ribonuclease, a low molecular weight protein, is assigned a value of 13,000–14,000.

4. *Alcohol-soluble proteins* (*prolamins*) are soluble in 70–80% ethyl alcohol but are insoluble in absolute alcohol and in water. Upon hydrolysis they yield large amounts of proline and amide nitrogen. Furthermore, they are deficient in lysine. Examples: gliadin (from wheat), hordein (from barley), zein (from corn), kafirin (kafir corn).

5. *Albuminoids* (*scleroproteins*) are very insoluble in all neutral solvents. They form the principal organic components of such external protective tissue as skin and hair and also of the skeletal structures of animals. Examples: keratin (from hair, nails, horn, and hoof), elastin (from connective tissue and ligament), collagen (from tendon).

6. *Histones* are soluble in water and insoluble in dilute ammonia. The coagulum obtained on heating is readily soluble in dilute acids and a precipitate is obtained when treated with solutions of other proteins. The basic amino acids predominate in their hydrolyzates. Because of the high percentage of diamino acids, they stand midway between the protamins and the remaining amino acids. Examples: globin (from hemoglobin), scombrone (from mackerel), thymus histone.

7. *Protamins* are less complex in structure than the proteins so far classified. They are soluble in water, cannot be coagulated by heat, are strongly basic in nature, and their hydrolyzates contain but few amino acids, arginine occurring in largest percentage. They precipitate aqueous solutions of other proteins and have the property of forming stable salts with strong mineral acids. Like the histones, they usually exist in combination with nucleic acids. Examples: salmine (from salmon sperm), sturine (from sturgeon sperm), clupeine (from herring sperm), cyprinine (from carp sperm), scombrine (from mackerel sperm).

2. Conjugated Proteins

Substances of this group are composed of protein combined with some nonprotein substance or substances (prosthetic group).

1. *Nucleoproteins* are compounds of one or more protein molecules with nucleic acid. Examples: thymonucleic acid (from thymus gland), cytoglobulin (from cytoplasm), nucleohistone (from nucleus), triticonucleic acid (from wheat germ).

2. *Glycoproteins* are compounds of the protein molecule with a substance or substances containing a carbohydrate group other than a nucleic acid. Examples: osseomucoid (from bone), tendomucoid (from tendon), mucin (from saliva), helicoprotein (from snail).

3. *Phosphoproteins* are compounds of the protein molecule with phosphorus-containing substances other than a nucleic acid or lecithin. Examples: casein (from milk), and possibly ovovitellin (from egg yolk).

4. *Chromoproteins* are compounds of the protein molecule with hematin or some substance similar in structure. Examples: hemoglobin (from red blood), helicorubin (from the liver and gut of *Helix pomatia*).

5. *Lecithoproteins* are compounds of the protein molecule with lecithin or some substance similar in structure. Example: lecithovitellin (from egg yolk).

3. Derived Proteins

The derived proteins are divided into primary and secondary protein derivatives formed through the hydrolytic cleavage of simple and conjugated proteins.

(a) *Primary Protein Derivatives*

These derivatives are products of hydrolytic cleavage which involve only slight changes in the properties of the original proteins.

1. Proteans are insoluble products which probably result from the incipient action of water, very dilute acids, or enzymes. Examples: casein (from coagulated milk), fibrin (from coagulated blood), myosan (from myosin), edestan (from edestin).

2. Metaproteins are produced by the further action of acids and alkalies. The molecule is changed to such an extent as to form products which are soluble in very weak acids and alkalies but insoluble in neutral solvents. Examples: acid metaprotein (acid albuminate), alkali metaprotein (alkali albuminate).

3. Coagulated proteins are produced by (*1*) the action of heat on protein solutions, or (*2*) the action of alcohols on proteins. Similar products have been produced by the action of mechanical shaking, ultraviolet light, x-rays, very high pressure, etc., on isoelectric solutions of proteins. Examples: proteins precipitated by the action of ethyl alcohol, heat coagulation of proteins in the process of cooking food (cooked egg albumin).

(*b*) *Secondary Protein Derivatives*

These are products of the further hydrolytic cleavage of the protein molecule. As hydrolysis of the protein molecule proceeds, it is broken up into fragments of varying sizes and properties. These fragments are named, respectively, proteoses, peptones, and peptides. These classes do not represent pure chemical substances but rather mixtures of fragments of the original protein. Relatively little is known of the individual substances composing these mixtures. For convenience, however, they are divided according to the concentration of ammonium or zinc sulfate required to precipitate them from solution.

1. Proteoses are soluble in water and are not coagulated by heat. These substances are precipitated by saturating their solutions with ammonium sulfate or zinc sulfate.

2. Peptones are soluble in water and are not coagulated by heat. They are not precipitated by saturating their solutions with ammonium sulfate or zinc sulfate but are precipitated by some other reagents (phosphotungstic acid, for example).

3. Peptides are compounds formed by the combination of two or more amino acids, the carboxyl group of one amino acid being united to the amino group of the other and the condensation having taken place with the elimination of water.

The relationship between the derived proteins may be indicated in the order of decreasing complexity of their molecules:

Protein → protean → metaprotein → proteose → peptone → peptides → amino acids

4. Conclusions

The classifications now in use are by no means adequate or final. Even the definition of the term *simple protein* is not entirely satisfactory. Thus, whereas egg albumin, serum albumin, and other proteins are classified as simple proteins, they have been shown to yield carbohydrates on hydrolysis.[7, 8] Classifications, based largely on solubility data, may lead to confusing quantitative results. Whey proteins, according to Osborne and Wakeman,[9] consist of 65% albumin, while Palmer[10] reports

---

[7] M. Sørensen and G. Haugaard, *Compt. rend. trav. lab. Carlsberg*, **19**, No. 12 (1933).

[8] M. Sørensen, *Compt. rend. trav. lab. Carlsberg, Sér. chim.*, **21**, 123 (1936).

[9] T. B. Osborne and A. J. Wakeman, *J. Biol. Chem.*, **33**, 7 (1918).

[10] A. H. Palmer, *J. Biol. Chem.*, **104**, 359 (1934).

60% in the form of globulin. Because of the tentative nature of the classifications, modifications have been suggested. Thus Meyer and Palmer[11] classify glycoproteins into mucins, mucoids, and glucosidoproteins, the first group being subdivided into mucins and sulfomucins. Furthermore, compounds have been isolated which do not fall readily into any of the suggested groups. In spite of the inherent weaknesses of the systems of classification in use, they serve well to bring about order in place of hopeless confusion.[11a]

III. ISOLATION OF PURE PROTEINS

In order to study the chemical, physical, and physicochemical properties of the proteins, it is essential to obtain them as pure as possible.[12-19] As found in nature, they are associated with many other materials. The methods which are applicable to the isolation of a particular protein are not determined by the chemical and physical nature of the protein alone but also by the source of the material and the substances from which it must be removed. The preparation of a pure protein, therefore, is not a simple matter. Even after the completion of the purification there may still remain an element of uncertainty concerning the composition of the preparation. Thus, for example, it has been shown that casein, gelatin, and protamins are not single chemical compounds but heterogeneous mixtures of several proteins.[20-22] This does not hold true for all proteins. Crystalline proteins, such as egg albumin and hemoglobin, probably are homogeneous.

In order to prepare purified proteins, such processes as extraction with solutions of sodium chloride or alcohol, precipitation at the isoelectric point of the protein, electrodialysis, dialysis, or fractional precipitation are used. All proteins, however, are not least soluble at their isoelectric point: for example, hemoglobin of man and sheep. The presence of autolytic enzymes in animal tissues requires quick freezing and drying procedures. The process of purification is further complicated if the source contains lipids. The latter must then be removed by using such solvents as acetone and ether.

---

[11] K. Meyer and J. W. Palmer, *J. Biol. Chem.*, **114**, *Proc. Am. Soc. Biol. Chem.* LXVIII (1936).

[11a] See R. J. Block, "The Chemical Constitution of the Proteins," in C. L. A. Schmidt, *The Chemistry of the Amino Acids and Proteins.* Thomas, Springfield, 1938.

[12] T. B. Osborne, *The Vegetable Proteins.* Longmans, Green, London, 1912.

[13] D. B. Dill and C. L. Alsberg, *J. Biol. Chem.*, **65**, 297 (1925); *J. Biol. Chem.*, **63**, *Proc. Am. Soc. Biol. Chem.* lxvii (1925).

[14] G. L. Taylor, G. S. Adair, and M. E. Adair, *J. Hyg.*, **32**, 340 (1932).

[15] W. C. Stadie and E. C. Rose, *J. Biol. Chem*, **68**, 229 (1926).

[16] G. S. Adair and M. E. Adair, *Biochem. J.*, **28**, 1230 (1934).

[17] M. L. Anson and A. E. Mirsky, *J. Gen. Physiol.*, **13**, 469 (1929–1930).

[18] D. M. Greenberg, *J. Biol. Chem.*, **78**, 265 (1928).

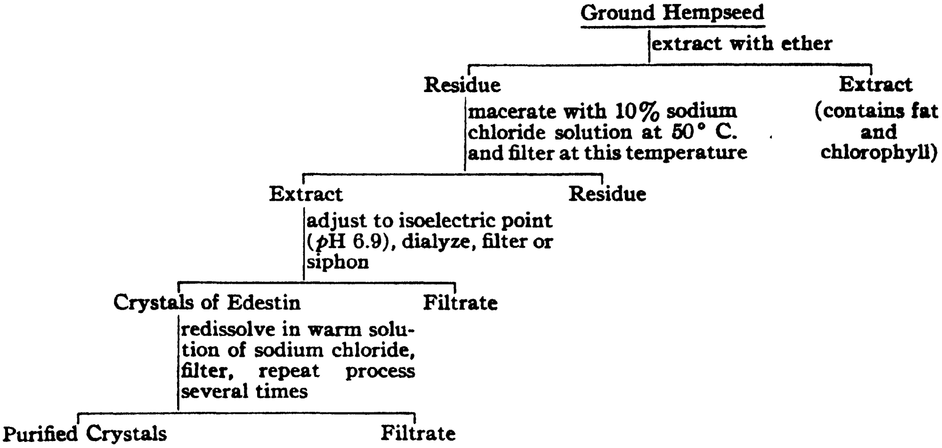
[19] E. J. Cohn, *Organic Syntheses.* Vol. 10, Wiley, New York, 1930, p. 16.

[20] T. Svedberg, L. M. Carpenter, and D. C. Carpenter, *J. Am. Chem. Soc.*, **52**, 241 and 701 (1930). D. C. Carpenter, *ibid.*, **53**, 1812 (1931); **57**, 129 (1935). E. Cherbuliez and F. Meyer, *Helv. Chim. Acta*, **16**, 600 (1933). J. Groh, E. Kardos, K. Denes, and V. Serenyi, *Z. physiol. Chem.*, **226**, 32 (1934).

[21] K. Krishnamurti and T. Svedberg, *J. Am. Chem. Soc.*, **52**, 2897 (1930). J. H. Northrop and M. Kunitz, *J. Gen. Physiol.*, **12**, 379 (1929).

[22] K. Felix and K. Dirr, *Z. physiol. Chem.*, **184**, 111 (1929). K. Felix and A. Lang, *ibid.*, **193**, 1 (1930).

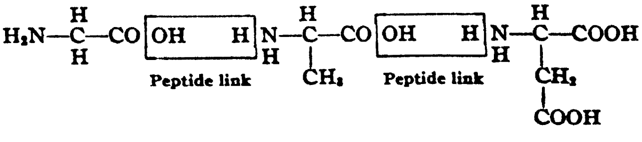
The principal steps involved in the procedure for the isolation of edestin, a typical plant protein, is indicated in the following flow sheet:


Ground Hempseed
extract with ether
Residue
macerate with 10% sodium chloride solution at 50° C. and filter at this temperature
Extract
(contains fat and chlorophyll)
Extract
adjust to isoelectric point (pH 6.9), dialyze, filter or siphon
Residue
Crystals of Edestin
redissolve in warm solution of sodium chloride, filter, repeat process several times
Filtrate
Purified Crystals
Filtrate

The ash content of the crystals may be reduced by the process of electrodialysis and the product may finally be dried by washing with alcohol, ether, and dehydrating over sulfuric acid.

IV. HYDROLYSIS OF THE PROTEINS

Protein molecules, as was stated before, consist of hundreds or even thousands of amino acids. The work of such investigators as Kossel, Fischer, Abderhalden, Osborne, Sørensen, Svedberg, Van Slyke, Bergmann, and others has given us some insight into the structural make-up of the protein molecules. Knowledge of the structure of the protein molecule has come from an examination of the nature of the products of hydrolysis and from the study of the physicochemical properties of the intact molecule. The older method of investigation informed us of the nature of the "building blocks" of these complexes; whereas physicochemical studies lead to a better understanding of the mode by which these units are combined in the unchanged protein molecule. The results of these investigations lead to the conclusion that the proteins consist of amino acids linked together by way of the carboxyl group of one and the amino group of the other with the simultaneous loss of water, and, further, that secondary valence forces play an important role in the formation of the characteristic patterns which are associated with the various proteins. The manner in which the amino acids are linked to form peptide chains may be indicated as follows:



$$H_2N{-}\overset{H}{\underset{H}{C}}{-}CO\underset{\text{Peptide link}}{\boxed{OH\quad H}}\underset{H}{N}{-}\overset{H}{\underset{CH_3}{C}}{-}CO\underset{\text{Peptide link}}{\boxed{OH\quad H}}\underset{H}{N}{-}\overset{H}{\underset{\underset{COOH}{CH_2}}{C}}{-}COOH$$

On hydrolytic cleavage, the peptide linkages present in the protein molecule are broken and the constituent amino acids are liberated. In this process, the original unit is broken down by way of proteoses, peptones, and peptides into the amino acids. Studies in connection with the action of proteolytic enzymes indicate that some amino acids are liberated more readily than others and that simple amino acids are split off from the protein molecules at the very beginning of the hydrolytic process.

The process of hydrolysis may be effected by (*a*) boiling with acids, (*b*) boiling with alkalies, (*c*) the action of proteolytic enzymes, and (*d*) heating for several hours in an autoclave (150° C.). Before discussing some of the advantages and disadvantages of the first three methods, it may be well to mention that every protein does not yield all the naturally occurring amino acids. This fact is illustrated in Table 11.

An examination of Table 11 reveals that it does not contain sufficient data for the more recently discovered amino acids, threonine and methionine; sufficient quantitative data for these constituents with relation to the many proteins listed are not as yet available. Furthermore, the technical difficulties involved in the quantitative determination of the products of hydrolysis do not permit attaining the theoretical summation values. Very often about two-thirds of the original substance is accounted for by way of quantitative determinations It must be emphasized at this point that the process of hydrolysis consists of the addition of water molecules, and complete recovery, therefore, would give a summation value of 110–120%. Whether the low quantitative values indicate the existence of unknown amino acids in some of the proteins or are due to the experimental procedures used, remains a question to be answered in the future. In the case of zein, however, it seems improbable that any new amino acid will be discovered.[23]

The particular procedure used for a hydrolysis depends upon the quantitative determinations which are to be made or upon the products which are to be isolated. It is not likely that the methods used to bring about the cleavage of the proteins into the constituent building blocks, the amino acids, and ammonia (together with the nonprotein prosthetic groups in the case of the conjugated proteins) completely accomplish their purpose.

The completeness of hydrolysis is often determined by applying the biuret test to both the solution and to any residue which may be left after the process. The biuret test is, however, not sufficient in many cases.[24] It is wise, therefore, to test the completeness of the hydrolytic processes by following the increase of ammonia and amino nitrogen with the Van Slyke amino nitrogen method or the Sørensen formol titration.

1. Acid Hydrolysis

Sulfuric acid was the first acid to be used to hydrolyze proteins. It is preferred by many investigators because it may readily be removed from the hydrolyzate with barium hydroxide.

The protein, when hydrolyzed with this acid, is usually boiled with six times its quantity of 35% sulfuric acid until the process is complete. The period of hydrolysis varies with the protein but is usually complete after 15 to 24 hours. Moreover, this acid is employed when Dakin's butyl alcohol

---

[23] H. B. Vickery, *Ind. Eng. Chem.*, **16**, 1029 (1924).

[24] T. B. Osborne and D. B. Jones, *Am. J. Physiol.*, **26**, 350 (1910).

TABLE 11

PERCENTAGES OF AMINO ACIDS FROM HYDROLYSIS OF VARIOUS PROTEINS[a]

Compound	Albumin (egg)	Albumin (serum)	Albumin (milk) lactalbumin	Albumin (wheat) leucosin	Globulin (serum)	Globulin (hemp) edestin	Keratin (hair)[b]	Keratin (wool)	Fibroin (silk)	Casein	Vitellin	Zein	Gliadin (wheat)	Hemoglobin	Gelatin	Bence-Jones protein	Salmine	Glutenin (wheat)	Gluten (wheat)	Histone (thymus)	Pepsin (crystalline)	Insulin (crystalline)
Glycine	0.0	0.0	0.4	0.9	3.5	3.8	4.7	0.6	40.5	0.5	1.1	0.0	0.0	..	25.5	1.7	..	0.9	0.4	0.5	..	..
Alanine	2.2	2.7	2.4	4.4	2.2	3.6	1.5	4.4	25.0	1.9	0.2	9.8	2.0	4.2	8.7	4.5	..	4.7	0.3	3.5	..	..
Valine	2.5	..	3.3	0.2	..	6.3	1.0	2.8	..	7.9	2.4	1.9	3.4	..	0.0	5.6	4.3	0.2	..	..	..	..
Leucine and isoleucine	10.7	20.0	14.0	11.3	18.7	14.5	7.1	11.5	2.5	9.7	11.0	25.0	6.6	29.0	7.1	10.6	..	6.0	4.1	11.8	..	30.0
Phenylalanine	5.1	3.1	1.3	3.8	3.8	3.1	..	..	11.5	3.9	2.8	7.6	2.4	4.2	1.4	4.9	..	2.0	1.0	2.2	..	..
Tyrosine	4.2	4.7	2.0	3.3	6.7	4.6	4.8	4.8	11.0	6.6	5.0	5.9	..	3.2	0.0	6.8	..	4.5	1.9	5.2	10.3	12.0
Tryptophane	1.3	0.5	2.7	1.2	2.3	2.5	1.8	1.8	..	2.2	1.3	0.2	1.1	1.3	0.0	2.5	..	1.7	..	..	2.2	..
β-Threonine	..	..	..	..	..	..	..	..	..	+	..	+	..	..	..	..	..	..	..	..	..	..
Glutamic acid	14.0	7.7	12.9	6.7	8.2	19.2	..	12.9	..	21.8	12.2	31.3	43.7	1.7	5.8	8.0	..	25.7	24.0	0.5	18.6	21.0
Hydroxyglutamic acid	1.4	..	10.0	..	..	..	..	..	..	10.5	..	2.5	2.4	..	0.0	..	..	1.8	..	..	..	..
Aspartic acid	6.1	3.1	9.3	3.3	2.5	10.2	..	2.3	..	4.1	0.5	1.8	0.8	4.4	3.4	2.2	..	2.0	0.7	..	6.8	..
Proline	4.2	1.0	3.8	3.2	2.8	4.1	3.4	4.4	1.0	9.0	4.0	9.0	13.2	2.3	9.5	2.7	11.0	4.2	4.0	1.5	..	..
Hydroxyproline	..	..	..	..	..	..	..	..	..	0.2	..	..	..	1.0	14.1	..	..	..	..	..	..	..
Serine	..	0.6	1.8	..	..	0.3	..	2.9	1.8	0.5	..	1.0	..	0.6	0.4	..	7.8	0.7	..	..	..	..
Hydroxyvaline	..	..	..	..	..	..	..	..	..	..	..	1.5	..	..	..	..	..	..	..	..	..	..
Cystine	1.3	6.1	4.3	1.5	1.0	1.0	18.9[b]	13.1	..	0.3	1.2	0.8	..	0.3	0.2	3.0	..	1.8	..	..	1.4	12.0
Methionine	4.6	..	2.6	..	..	2.1	..	..	..	3.4	..	2.3	2.1	..	0	..	..	..	..	..	..	..
Arginine	5.2	4.8	3.0	5.9	5.2	15.8	8.0[b]	7.8	0.7	3.8	7.8	1.8	2.9	3.1	8.2	5.1	87.4	4.7	4.4	27.1	2.7	3.0
Histidine	1.4	1.2	2.1	2.8	0.9	2.2	0.5[b]	0.7	0.1	2.5	1.2	0.8	1.5	7.6	1.0	1.1	..	1.8	1.2	5.8	0.1	8.0
Lysine	6.4	6.9	8.8	2.7	6.2	2.2	2.5[b]	2.3	0.3	6.0	5.4	0.0	0.6	8.1	5.9	6.8	..	1.9	2.2	9.7	2.1	2.0
Ammonia	1.4	..	1.3	1.4	..	2.3	..	..	..	2.3	..	3.6	5.2	..	0.4	5.6	..	4.0	..	7.5	8.8	..
Total	72.0	62.4	86.0	52.6	64.0	97.8	54.2	72.3	84.4	97.1	56.1	106.8	87.9	71.0	91.6	71.1	110.5	68.6	44.2	75.3	53.0	88.0

[a] C. L. A. Schmidt, *The Chemistry of the Amino Acids and Proteins.* Thomas, Springfield, 1938, p. 217.

[b] Human hair.

extraction is used or the isolation of basic amino acids is required. Hydrochloric acid hydrolysis, which is preferable when monoamino acids are the subject of investigation, is usually carried out by boiling the protein with three times the quantity of the concentrated acid, or with 10 to 20 parts of dilute (20%) hydrochloric acid. Complete hydrolysis may be obtained by boiling the protein for a period of $1^1/_2$ to 2 hrs. in an autoclave at 150° C. The time required for the usual hydrochloric acid procedure is from 6 to 20 hours, although in certain cases (*e. g.*, casein) longer periods may be required. Van Slyke[25] has shown that the amino nitrogen value reaches a maximum and then, on further hydrolysis, decreases slowly because of the slow destruction of the amino acids. In this connection it must be mentioned that hydrolysis with acids brings about almost complete destruction of tryptophane.

Other acids, such as hydrofluoric, formic, and acetic, have also been used, but they offer no definite advantages. Some investigators recommend that hydrolysis in an autoclave be carried out with dilute acids; this procedure, it is claimed, does not lead to the formation of the brownish-black precipitate (humin or melanin).

When proteins are hydrolyzed by means of acids, a dark-colored precipitate called humin or melanin is formed. Numerous investigations by Gortner and his coworkers[26] indicate that humin is formed by condensation of the indole nucleus of tryptophane with an aldehydic constituent present in the protein molecule. Other amino acids, *e. g.*, tyrosine, may be involved in the formation of this product. Furthermore, the formation of insoluble humin involves other reactions, such as oxidation and molecular rearrangement. Obviously the formation of this substance interferes with the quantitative determination of some of the amino acids, particularly tryptophane. The formation of the soluble form of humin involves a loss of tyrosine.

2. Alkaline Hydrolysis

Alkalies, such as sodium hydroxide and potassium hydroxide, effect very rapid hydrolysis. Nevertheless, alkaline hydrolysis is not generally used because it causes complete racemization of the amino acids and completely decomposes arginine and cystine. Tyrosine and tryptophane are not decomposed by boiling solutions of alkalies and these amino acids may be determined quantitatively in the hydrolyzates.

3. Hydrolysis with Proteolytic Enzymes

The use of proteolytic enzymes,[27-30] occurring in animal and plant tissues, forms the basis of another method of cleaving the proteins hydrolytically. The studies undertaken by many investigators indicate that the action of proteolytic enzymes is slow and incomplete. Nevertheless, hydrolysis by means of enzymes has been of great value in the isolation of amino acids from proteins.[31–33] These agents act only at relatively low temperatures and in slightly alkaline or acid media. Such gentle treatment is less likely to alter or destroy the constituent building blocks. As no racemization takes place, the amino acids may be isolated in the same optical form they possess in the original protein.

---

[25] D. D. Van Slyke, *J. Biol. Chem.*, **12**, 295 (1912).

[26] R. A. Gortner, *Outlines of Biochemistry*. Wiley, New York, 1929

[27] C. Oppenheimer, *Die Fermente und ihre Wirkungen*. Vol. 2, G. Thieme, Leipzig, 1925, Lieferung 6-13.

[28] J. M. Luck, *Annual Review of Biochemistry*. Vols. I-XI, Annual Reviews, Inc., Stanford Univ., Calif., 1932-1942.

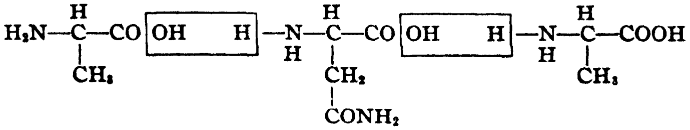
[29] H. O. Calvery, *J. Biol. Chem.*, **102**, 73 (1933).

[30] J. H. Northrop, *J. Gen. Physiol*. **4**, 57 (1921-1922).

Ammonia, another compound found in protein hydrolyzates, is formed mainly by the hydrolysis of the acid amide group:

$$-CONH_2 + H_2O \longrightarrow -COOH + NH_3$$

The carboxyl group of dicarboxylic amino acids which is not involved in the formation of peptide linkages is found mainly in the acid amide form. While a small amount of ammonia may be formed by the action of acids on the monoamino acids, by far the largest amount is due to the hydrolysis of the acid amide group. The existence of acid amide groups in peptide chains may be indicated as follows:



$$H_2N-\overset{H}{\underset{CH_3}{C}}-CO\boxed{OH\quad H}-\underset{H}{N}-\overset{H}{\underset{\underset{CONH_2}{CH_2}}{C}}-CO\boxed{OH\quad H}-\underset{H}{N}-\overset{H}{\underset{CH_3}{C}}-COOH$$

V. CLASSIFICATION OF THE NATURALLY OCCURRING AMINO ACIDS

Vickery and Schmidt[34] suggest that only those amino acids which have been found by more than one investigator in more than one protein should be included in classifications dealing with the naturally occurring amino acids. Names of the discoverers as well as the dates of discovery of these amino acids are indicated by these authors.

With certain additions and modifications, the major subdivisions of the classification which follows are based on those suggested by H. B. Vickery.[35]

A. Amino acids concerning which there is no doubt whatever:

1. Aliphatic Amino Acids
   (*a*) Monoaminomonocarboxylic acids
      Glycine (aminoacetic acid)

$$CH_2(NH_2)-COOH$$

Alanine (α-aminopropionic acid)

$$CH_3-CH(NH_2)-COOH$$

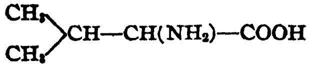
Serine (α-amino-β-hydroxypropionic acid)

$$CH_2OH-CH(NH_2)-COOH$$

---

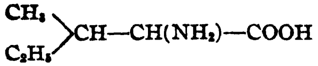
[31] K. Felix, *Z. physiol. Chem.*, **146**, 103 (1925).
[32] F. Lieben and H. Lieber, *Biochem. Z.*, **275**, 38 (1934).
[33] H. O. Calvery and E. D. Schock, *J. Biol. Chem.*, **113**, 15 (1936).
[34] H. B. Vickery and C. L. A. Schmidt, *Chem. Revs.*, **9**, 169 (1931).
[35] H. B. Vickery, *Ann. N. Y. Acad. Sci.*, **41**, 90 (1941).

Valine (**α-aminoisovaleric acid**)



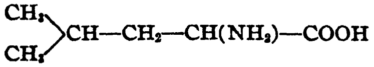
$$(CH_3)_2{>}CH—CH(NH_2)—COOH$$

Isoleucine (**α-amino-β-methyl-β-ethylpropionic acid**)



$$\begin{matrix} CH_3 \\ C_2H_5 \end{matrix}{>}CH—CH(NH_2)—COOH$$

Leucine (**α-aminoisocaproic acid**)



$$(CH_3)_2{>}CH—CH_2—CH(NH_2)—COOH$$

Threonine (**α-amino-β-hydroxy-*n*-butyric acid**)

$CH_3—CHOH—CH(NH_2)—COOH$

(*b*) Monoaminodicarboxylic acids

Aspartic acid (aminosuccinic acid)

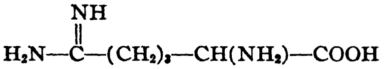
$HOOC—CH_2—CH(NH_2)—COOH$

Glutamic acid (α-aminoglutaric acid)

$HOOC—CH_2—CH_2—CH(NH_2)—COOH$

(*c*) Diaminomonocarboxylic acids

Arginine (α-amino-δ-guanidinovaleric acid)



$$H_2N—\overset{\overset{\displaystyle NH}{\|}}{C}—(CH_2)_3—CH(NH_2)—COOH$$

Lysine (α-amino-ε-aminocaproic acid)

$CH_2(NH_2)—(CH_2)_3—CH(NH_2)—COOH$

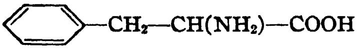
(*d*) Sulfur-containing acids

Methionine (α-amino-γ-methylthiol-*n*-butyric acid)

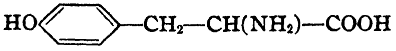
$CH_3—S—CH_2—CH_2—CH(NH_2)—COOH$

2. Aromatic Amino Acids

Phenylalanine (α-amino-β-phenylpropionic acid)

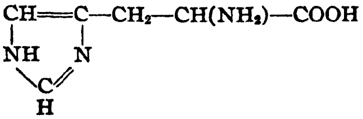


Tyrosine (α-amino-β-*p*-hydroxyphenylpropionic acid)

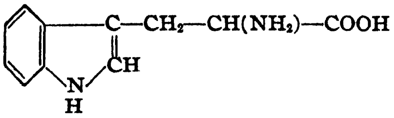


3. Heterocyclic Amino Acids

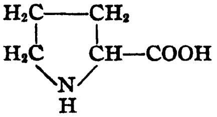
Histidine (α-amino-β-imidazolepropionic acid)



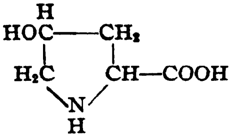
Tryptophane (α-amino-β-3-indolepropionic acid)



Proline (α-pyrrolidine carboxylic acid)



Hydroxyproline (γ-hydroxy-α-pyrrolidine carboxylic acid)



B. Amino acids that occupy a special position because of limited distribution or for other reasons:

1. Aliphatic Amino Acids
   - (*a*) Monoaminomonocarboxylic acid

     Norleucine (α-amino-*n*-caproic acid)

     $CH_3—CH_2—CH_2—CH_2—CH(NH_2)—COOH$

     Found in the spinal chord protein, neuroplastin, and nerve tissue.
   - (*b*) Monoaminodicarboxylic acid

     β-Hydroxyglutamic acid (α-amino-β-hydroxyglutaric acid)

     $HOOC—CH_2—CHOH—CH(NH_2)—COOH$

     Existence in proteins doubted by some investigators.
   - (*c*) Sulfur-containing amino acids

     Cystine (di-α-amino-β-thiopropionic acid)

     $HOOC—CH(NH_2)—CH_2—S—S—CH_2—CH(NH_2)—COOH$

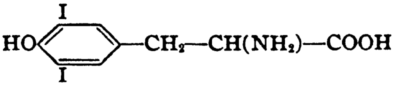
     Universally distributed; mode of linkage of the sulfur atoms still under discussion.

     Cysteine (α-amino-β-thiopropionic acid)

     $HS—CH_2—CH(NH_2)—COOH$

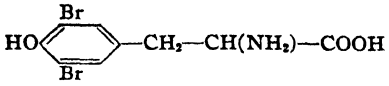
     Nitroprusside reaction points to the possible presence in some native proteins.
2. Aromatic Amino Acids

   Diiodotyrosine (α-amino-β-*p*-hydroxy-3,5-diiodophenylpropionic acid)



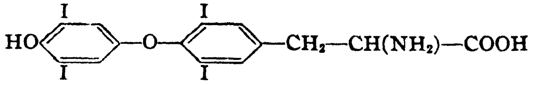
Found in the proteins of the thyroid gland and skeleton protein of some marine organisms: also known by the name of iodogorgoic acid.

Dibromotyrosine (α-amino-β-*p*-hydroxy-3,5-dibromophenylpropionic acid)



Found in the horny skeleton of the coral, *Primioa lepalifera.*

Thyroxine (β-[3,5-diiodo-4-(3′,5′-diiodo-4′-hydroxyphenoxy)-phenyl]-α-aminopropionic acid)



Found in the proteins of the thyroid gland.

C. **Amino acids which have been found in plant constituents and which may possibly be expected in proteins:**

Canavanine (α-amino-γ-guanidinoxy-*n*-butyric acid)

$$\mathrm{H_2N{-}C(:NH){-}NH{-}O{-}CH_2{-}CH_2{-}CH(NH_2){-}COOH}$$

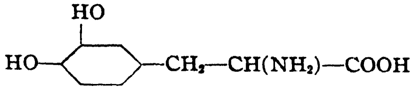
Found in soybean meal.

Citrulline (δ-carbaminoornithine)

$$\mathrm{H_2N{-}CO{-}NH{-}CH_2{-}CH_2{-}CH_2{-}CH(NH_2){-}COOH}$$

Found in watermelon tissue.

Dihydroxyphenylalanine (α-amino-β-3,4-dihydroxyphenylpropionic acid)



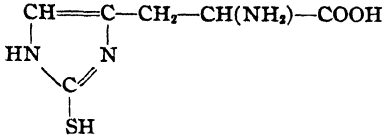
Found in seedlings of velvet beans and in sprouts of velvet beans and pods of *Vicia faba.*

Djenkolic acid (cysteine thioacetal of formaldehyde)

$$\mathrm{HOOC{-}(H_2N)HC{-}CH_2{-}S{-}CH_2{-}S{-}CH_2{-}CH(NH_2){-}COOH}$$

Found in djenkol nuts.

Thiolhistidine (α-amino-2-thiol-β-imidazolepropionic acid)



Found as the betaine of thiolhistidine, ergothionine, in red blood corpuscles and ergot.

D. Amino acids which have been reported but whose presence in proteins lacks confirmation:

Aminobutyric acid
Base ($C_{14}H_{11}O_8N$)
Caseianic acid
Caseinic acid
Diaminoadipic acid
Diaminoglutaric acid
Diaminotrihydroxydodecanic acid
Dihydroxydiaminosuberic acid
Dodecandiaminodicarboxylic acid
Hydroxyaspartic acid
Hydroxylysine
Hydroxytryptophane
Hydroxyvaline
Hyphasamine ($C_{16}H_{24}O_5N_2$)
Norvaline
Prolysine
Protoctine ($C_8H_{15}O_3N_3$)

The existence of additional amino acids has been reported but the experimental evidence for their existence in proteins is of such doubtful nature that they are not included in the above list.

VI. ISOLATION OF THE AMINO ACIDS

The separation and isolation of the amino acids from the hydrolyzates of proteins involve chemical and physical procedures which vary according to the particular separation to be made or the particular amino acids to be isolated. While details of experimental procedures cannot be considered in this treatment, some of the principles involved will be briefly discussed.

The reagents used to bring about the decomposition of proteins into their constituent building blocks, the α-amino acids, have already been enumerated (see page 115).

The agents which bring about the hydrolytic cleavage of proteins during the processes of digestion *in vivo* are called proteolytic enzymes (the proteases). These enzymes have been found to be more or less specific in their action. This specificity of the action of the proteolytic enzymes is best explained by assuming that there are various types of linkages between the amino acids making up the protein molecule. Abderhalden[36] assumes the presence of anhydride and ester linkages, diketopiperazine rings, as well as the acid amide union. It is not within the scope of this treatment to consider the physicochemical and colloidal aspect of enzyme action. It may be stated, however, that many investigations have been carried on in order to study the selective liberation of amino acids by the action (*in vitro*) of enzymes on proteins. Whereas early investigations by Abderhalden indicated that free amino acids do not result from the action of pepsin on proteins, more recent experimental observations point in the opposite direction.[37-39] Comparative studies of the extent of hydrolysis by means of acids, alkalies, or enzymes have been carried out particularly by such investigators as Northrop,[40] and Calvery and collaborators.[41] Concerning the conclusions we may draw from these experiments, a quotation from Calvery (Schmidt, *The Chemistry of the Amino Acids and Proteins*. Thomas, Springfield, 1938, p. 138) is appropriate, "Summarizing: Proteins are hydrolyzed by the three groups of hydrolytic agents at different rates and to different extents; some amino acids are liberated almost completely very early by any one or all three of the hydrolytic agents, while some form very resistant groupings in the protein molecule. Tyrosine, cystine, and arginine are among those amino acids which are readily liberated, while proline and phenylalanine are among the resistant ones."

---

[36] E. Abderhalden, *Z. physiol. Chem.*, **128**, 119 (1923); **129**, 106 (1923).

[37] E. Abderhalden, *Lehrbuch der Physiologischen Chemie*. Part I, Urban and Schwarzenberg. Berlin, 1923.

[38] J. H. Northrop, *J. Gen. Physiol.*, **13**, 739 (1930).

[39] H. O. Calvery and E. D. Schock, *J. Biol. Chem.*, **113**, 15 (1936).

[40] J. H. Northrop, *J. Gen. Physiol.*, **4**, 57 (1921-1922).

[41] H. O. Calvery, W. D. Block, and E. D. Schock, *J. Biol. Chem.*, **113**, 21 (1936).

The isolation of the various amino acids found present in protein hydrolyzates is often preceded by their separation into groups. Several methods have been developed to bring about such preliminary separations.

Fischer's Ester Method.—This method, as originally proposed by Fischer,[42] was to be used for the qualitative rather than the quantitative separation of the amino acids. Modifications introduced by various investigators[43–47] increase the value of this procedure for quantitative work.

The method, very briefly, consists in the esterification of the protein hydrolyzate with ethyl alcohol and hydrogen chloride. The free esters, after being liberated from the ester hydrochlorides by treatment with alkali, are fractionally distilled under reduced pressure. The following results, taken from Plimmer, *The Chemical Constitution of the Proteins*, Part I, Longmans, Green, London, 1917, p. 42, indicate the composition of the fractions usually collected:

Fractions	Temperature, ° C.	Pressure, mm.	Esters of
I	To 60 (vapor)	10	Glycine, alanine, leucine, proline
II	60-90 (vapor) 100 (water bath)	10	Valine, leucine, proline
III	100 (water bath)	0.5	Leucine, proline
IV	100-160 (oil bath)	0.5	Phenylalanine, glutamic acid, aspartic acid, serine

This method and the many modifications which have improved the original procedure are beset with many technical difficulties and the results are far from quantitative.

Cherbuliez' Ester Method.—The protein hydrolyzate is treated with phosphotungstic acid, in order to precipitate the basic amino acids. After removal of the phosphotungstic acid, the amino acids are esterified by means of absolute alcohol and dry hydrogen chloride. The ester hydrochlorides are converted into the acetyl ethyl esters of the amino acids by acetylation with acetic anhydride in the presence of sodium acetate. The acetyl esters are then removed by means of chloroform extraction and eventually separated by fractional distillation under reduced pressure.[48]

Dakin's Butyl Alcohol Extraction.—The protein is hydrolyzed with sulfuric acid and the acid is then quantitatively removed by barium hydroxide. The resulting solution is concentrated to a sirup and subjected to extraction with butyl alcohol. The treatment with butyl alcohol is best carried out with a continuous extractor and under reduced pressure. In this manner the monoamino monocarboxylic acids as well as proline are extracted by the butyl alcohol. If the process is carried out at atmospheric pressure, hydroxyproline is also removed. The butyl alcohol is then removed from the extract and the residue extracted with absolute alcohol. Proline is soluble in the absolute ethyl alcohol, hence this procedure forms the basis for a valuable method for the isolation of proline. The residue not extracted by ethyl alcohol may be separated into the component amino acids by the application of other methods, such as Fischer's ester method. The amino acids not extracted by butyl alcohol consist mainly of the dicarboxylic and basic amino acids. The two classes may be separated by precipitating the basic amino acids with the help of phosphotungstic acid. Finally, the dicarboxylic amino acids can be removed as the barium salts.

This method was first developed by Dakin[49] while attempting to isolate optically active proline.

---

[42] E. Fischer, *Z. physiol. Chem.*, **33**, 151 (1901).

[43] T. B. Osborne and D. B. Jones, *Am. J. Physiol.*, **26**, 212 (1910).

[44] N. Zelinsky, A. Annenkoff, and J. Kulikoff, *Z. physiol. Chem.*, **73**, 459 (1911).

[45] F. W. Foreman, *Biochem. J.*, **13**, 378 (1919).

[46] B. O. Pribram, *Monatsh.*, **31**, 51-54 (1910).

[47] P. A. Levene and D. D. Van Slyke, *J. Biol. Chem.*, **6**, 419 (1909).

[48] E. Cherbuliez, P. Plattner, and S. Ariel, *Helv. Chim. Acta*, **13**, 1390 (1930)

[49] H. D. Dakin, *Biochem. J.*, **12**, 290 (1918); *J. Biol. Chem.*, **44**, 499 (1920).

During this process of extraction the individual amino acids retain their optical activity, and the method, therefore, is particularly valuable for the preparation of amino acids which are to be used for metabolism experiments. This method, furthermore, permits a relatively high degree of quantitative accuracy when used in connection with protein analysis and the isolation of certain amino acids (*i. e.*, proline, tryptophane, and others). Dakin, using the butyl alcohol procedure and Foreman's method[60] for the determination of dibasic amino acids, was led to the discovery of β-hydroxyglutamic acid.

The Electrical Transport Method.—On passing a direct current through a protein hydrolyzate placed in the center of a three-compartment electrolysis cell, the amino acids separate into three fractions. If the reaction of the middle compartment is properly adjusted, *p*H 5.5, the amino acids which are predominantly acid, including aspartic acid, glutamic acid, and methionine, migrate to the anode and the basic amino acids, arginine, histidine, and lysine, migrate to the cathode; the monoamino monocarboxylic acids, which are not appreciably dissociated at a *p*H of 5.5, remain in the center compartment. Further separation may be brought about by adjusting the *p*H of the solution containing arginine, histidine, and lysine to a *p*H of 7.5, when, upon repetition of the electrical transport method, arginine and lysine migrate to the cathode, leaving the histidine in the center compartment. This method has thus far been used mainly in connection with the qualitative separation of amino acids; further progress along quantitative lines is desirable. This method is most useful for the isolation of amino acids such as glutamic acid, β-hydroxyglutamic acid, histidine, and arginine.[61-64]

TABLE 12

SOME PRECIPITANTS OF AMINO ACIDS[a]

Precipitant	Amino acid precipitated
Flavianic acid	Arginine histidine
Picric acid	Arginine, histidine, lysine, proline, glycine
Picrolinic acid	Basic amino acids, monoaminomonocarboxylic acids (particularly tryptophane and phenylalanine but not proline and hydroxyproline)
Reinecke salt	Proline, hydroxyproline
Phosphotungstic acid	Basic amino acids, cystine
Mercuric sulfate	Tryptophane, histidine
Silver sulfate	Arginine, histidine, glutamic acid, aspartic acid, β-hydroxyglutamic acid
Calcium hydroxide	Glutamic acid, aspartic acid
Barium hydroxide	Glutamic acid, aspartic acid
Zinc acetate or zinc chloride	Glutamic acid, histidine, hydroxyvaline, phenylalanine, leucine
Cuprous oxide	Cystine

[a] The list of precipitants and amino acids precipitated is by no means complete; however, it does include reagents which have been used for the quantitative removal of the amino acids.

Brazier's Method for the Fractionation of the Copper Salts of the Amino Acids.—The principle of this method is the preparation of the copper salts by heating an aqueous solution of the amino acids with copper carbonate. The salts are then dried thoroughly with acetone, and powdered. The copper salts, showing different solubility in water and in methyl alcohol, are separated into the following fractions: (*a*) copper salts insoluble in water (phenylalanine, leucine, and aspartic acid); (*b*) copper salts soluble in water but insoluble in methyl alcohol (alanine, glycine, tyrosine, lysine, arginine, histidine, and glutamic acid); (*c*) copper salts which are soluble in methyl alcohol (valine, hydroxyvaline, and proline). Further separation involves preparation of the metallic salts of some

---

[60] F. W. Foreman, *Biochem. J.*, 8, 463 (1914).

[61] K. Ikeda and S. Suzuki, U. S. Patent 1,015,891 (Jan. 30, 1912).

[62] G. L. Foster and C. L. A. Schmidt, *J. Biol. Chem.*, 56, 545 (1923).

[63] J. M. Gulland and C. J. O. R. Morris, *J. Chem. Soc.*, 1934, 1644.

[64] A. A. Albanese, *J. Biol. Chem.*, 134, 467 (1940).

TABLE 13

THE APPROXIMATE AMINO ACID COMPOSITION OF SOME PROTEINS[a]

Protein	Nitrogen, %	Sulfur, %	Cystine, %	Methionine,[b] %	Arginine,[c] %	Histidine,[c] %	Lysine,[c] %	Tyrosine, %	Tryptophane, %	Phenylalanine, %	Threonine, %	Valine, %	Leucine, %	Isoleucine, %
Brain	14.1	1.1	1.5	2−	6.5	2.1	6.4	3.7	1.1	5.2	5.2	5	10	4
Serum	14.9	1.5	3.3	2	5.9	2.3	7.8	4.1	1.4	7.1	5.7	7	14	3
Seralbumin	15.7	1.77	5	...	6.2	2.8	8.5	4.3	1.0	7.8	5.6	4	15	2
Serum globulin	14.2	1.0	2.0	2	5.2	1.8	5.8	5.2	2.0	5.9	6.0	...	..	...
Milk (purified)	15.2	0.95	1.1	3+	4.3	2.4	6.6	5.2	1.3	6.0	4.2	6	12	5
Casein	14.7	0.66	0.3	3	4.3	1.9	6.9	5.2	1.2	5.8	4.6	6	12	5
Lactalbumin	13.6	1.3	3.1	3−	3.0	1.4	7.9	3.4	2.0	4.8	4.5	5	15	5
Egg, whole	14.0	1.5	1.9	5−	6.4	1.8	5.0	3.7	1.4	5.0	4.2	5	12	5
Egg, albumin	13.9	1.6	1.7	5	5.6	1.5	5.0	3.5	1.4	6.8	3.6	5	10	5
Egg, yolk	14.6	1.1	1.9	3	7.7	2.3	5.0	4.0	1.5	4	4.4	...	..	...
Gelatin	16.0	0.2	0	1−	9.1	0.4	4.6	0	0	2.6	1.2	2.3	3.3	1.0
Neurogelatin	14.7	1.5	0	...	6.9	0.8	4.9	3.3	0.8	...	...	...	..	...
Muscle	16.1	..	1.1	3.0	7.7	1.7	6.9	3.4	1.1	4.9	3.5	4	11	3
Heart	14.8	..	1.1	...	6.8	2.3	7.9	3.6	1.2	5.3	3.7	3	13	4
Tripe	15.3	..	1.2	...	8.9	1.7	6.5	2.9	0.5	4.8	3.3	3	10	3
Bladder	16.0	..	1.1	...	7.6	1.3	5.6	...	...	3.8	3.3	...	..	...
Liver	13.3	0.94	1.2	...	6.0	2.0	4.6	3.0	1.0	6.2	4.6	4	10	4
Kidney	15.6	1.0	1.4	...	7.2	1.8	5.6	2.7	1.2	5.5	4.4	4	10	4
Lung	15.3	0.9	1.4	...	7.3	2.3	4.6	3.0	0.8	5.5	3.6	3	10	3
Spleen	15.7	..	1.2	...	7.2	1.8	6.8	2.3	0.6	4.5	3.7	5	10	3–4
Thymus	15.4	0.75	1.1	...	8.3	2.1	6.6	2.4	0.7	3.2	3.9	3	9	3
Salivary gland	15.7	..	1.0	...	7.5	2.6	6.4	2.7	0.8	3.6	3.4	...	..	...
Testes	15.4	..	1.2	...	7.5	1.7	6.0	2.7	0.9	5.3	3.4	...	..	...
Ovaries	15.8	..	1.2	...	7.5	1.5	5.3	2.5	0.6	4.8	3.6	...	..	...
Pancreas	15.5	..	1.5	...	6.8	2.5	5.5	2.5	1.2	4.3	4.0	4	9	3

TABLE 13 (*Continued*)

THE APPROXIMATE AMINO ACID COMPOSITION OF SOME PROTEINS

Protein	Nitrogen, %	Sulfur, %	Cystine, %	Methionine,[b] %	Arginine,[c] %	Histidine,[c] %	Lysine,[c] %	Tyrosine, %	Tryptophane, %	Phenylalanine, %	Threonine, %	Valine, %	Leucine, %	Isoleucine, %
Yeast	7.9	0.46	0.5	2−	2.4	1.4	3.4	2.2	0.6	2.2	2.7	3	6	2
Yeast	14.4	0.61	1	2−	3.7	4.7	4.7	3.2	1.3	3.9	2.7	...	..	...
Yeast	7.0	0.40	0.4	1+	1.9	1.1	3.0	1.9	0.5	1.7	2.1	2	5	1–3
Yeast	7.8	0.40	0.4	1+	2.4	1.4	3.6	2.3	0.6	2.0	2.2	2	4	2
Yeast	9.7	0.48	0.7	1+	3.2	1.9	4.3	2.4	0.8	2.0	...	...	..	...
Wheat gluten	13.1	0.87	1.5	2	2.8	1.4	1.6	3.3	0.7	4.5	2.2	3	7	2
Wheat gluten	11.9	0.79	1.5	2	2.9	1.6	1.3	2.9	0.5	4.3	2.0	3	7	3
Wheat gluten	12.4	0.93	1.5	2+	3.1	1.7	1.5	2.8	0.5	4.3	2.1	3	8	3
Wheat gluten	12.9	0.92	1.5	2+	2.7	1.5	1.5	3.0	0.5	4.3	2.1	3	8	3
Wheat gluten	13.0	0.86	1.5	2	2.7	1.6	1.3	3.0	0.4	4.5	2.1	3	7	3
White bread	11.2	0.80	1.6	2−	2.4	1.5	2.0	3.3	1.1	3.8	2.0	3	5	2
White bread	11.3	0.83	1.5	2	2.3	1.4	1.8	3.0	0.8	3.4	1.9	2	7	2
Yellow corn	8.8	0.95	0.7	3+	2.8	1.9	...	3.0	0.6	2.2	2.0	3	9	1–2
Yellow corn	7.6	0.86	0.8	3	1.7	1.1	1.0	2.6	0.7	1.8	1.4	2	10	1–2
Corn albumins	12.6	1.5	0.4	...	4.8	4.3	...	2.6	0.8	1.2	2.5	2	5	1
Corn albumins	12.0	2.2	0.6	...	3.5	5.0	0.7	3.8	0.6	1.5	3.0	3	8	1
Corn albumins	13.3	1.75	...	...	4.5	5.1	1.5	2.6	...	1.3	3.9	2	5	1
Corn albumins	7.7	1.1	0.2	...	...	...	...	...	...	...	...	2	3	1
Corn gluten	12.7	1.2	1.2	2−	2.5	1.1	0.5	5.3	0.6	5.1	3.0	4.0	23.9	3.7
Corn germ	4.4	0.4	0.4	1	1.3	0.7	1.2	1.9	0.5	1.2	1.0	2	3	1
Corn germ	12.8	0.81	1.0	3−	7.8	2.3	5.2	3.6	1.2	4.4	3.2	5	9	3
Soybean	8.1	0.66	0.7	2	3.3	1.1	3.0	2.1	0.8	2.9	2.1	2	4	2–3

[a] R. J. Block and D. Bolling, *The Determination of the Amino Acids.* Burgess, 1940; data revised by Dr. R. J. Block, October, 1943.

[b] Calculated from total S less cystine S. Approximate only.

[c] Corrected for mechanical losses.

of the amino acids (*i. e.*, barium salts of glutamic acid and aspartic acid; zinc salts of phenylalanine, leucine, histidine, and hydroxyvaline), formation of the flavianate of arginine, extraction with butyl alcohol and absolute alcohol, and numerous fractional crystallizations. Recent studies on the use of copper salts for the separation of amino acids have been carried out by Brazier[55] and by Town.[56]

The Use of Organic and Inorganic Reagents.—As noted above, the final isolation of the individual amino acids is often accomplished by the use of specific solvents, fractional crystallization, preparation of insoluble derivatives, and a combination of two or all of these methods. Many organic and inorganic compounds have been used as reagents for the formation of insoluble derivatives of specific amino acids. When prepared in conjunction with the standard preliminary methods for the separation of amino acids, these derivatives may be used in the quantitative determination or isolation of particular amino acids. A few of these reagents and some of the amino acids they precipitate are listed in Table 12.

Investigators who wish to prepare a particular amino acid must use good judgment in selecting the protein from which the substance is to be isolated.

An examination of Tables 11 and 13 shows a wide variation in the quantitative composition of the proteins. Thus, the vegetable proteins are low in cystine content, gliadin is a good source for glutamic acid, and gelatin lacks cystine, tryptophane, and tyrosine but is rich in glycine. Zein of corn is lacking in glycine, lysine, and tryptophane. As has been pointed out in the section dealing with the classification of proteins, albumins are often wanting in glycine whereas globulins usually contain this amino acid; prolamins yield large amounts of proline; the histones are rich in basic amino acids; protamins, such as salmine, are excellent sources for arginine; keratin, an albuminoid, is very rich in cystine; silk waste, casein, human hair, gluten of wheat flour, blood corpuscle paste, silk fibroin, and the thyroid gland are good starting materials for the preparation of *l*-tyrosine, *l*-tryptophane or *l*-tyrosine, *l*-cystine, *d*-glutamic acid, *l*-histidine, *d*-alanine, and thyroxine, respectively. Detailed procedures for the isolation of several amino acids are found in the following references: glycine,[57] *l*-cystine,[58] *d*-glutamic acid,[59] *l*-methionine,[60] *l*-tyrosine,[61] *l*-tryptophane,[62] *l*-threonine,[63] thyroxine.[64]

VII. SYNTHETIC METHODS

As is the case with most of the biochemically active substances found in the plant and animal world, the amino acids (with the exception of proline and alanine) were isolated from natural sources before methods for their synthesis became available. The structure of the natural product was eventually determined when its artificial production was achieved. Synthetic methods for the production of amino acids have, aside from academic interest, particular value in the case of amino acids which are difficult to isolate from natural sources. In the case of many of the amino acids, large quantities are more readily produced by synthetic than by isolation procedures.

---

[55] M. A. B. Brazier, *Biochem. J.*, **24**, 1188 (1930).

[56] B. W. Town, *Biochem. J.*, **30**, 1837 (1936).

[57] M. Bergmann and S. W. Fox, *J. Biol. Chem.*, **109**, 317 (1935).

[58] R. A. Gortner and W. F. Hoffman, *Organic Syntheses.* Vol. 5, Wiley, New York, 1925, p. 39.

[59] H. B. Lewis, H. M. Chiles, and G. J. Cox, *Organic Syntheses.* Vol. 5, Wiley, New York, 1925, p. 63.

[60] N. W. Pirie, *Biochem. J.*, **26**, 1270 (1932).

[61] P. B. Hawk and O. Bergeim, *Practical Physiological Chemistry.* Blakiston, Philadelphia, 1937.

[62] G. J. Cox and H. King, *Organic Syntheses.* Vol. 10, Wiley, New York, 1930, p. 100.

[63] R. H. McCoy, C. E. Meyer, and W. C. Rose, *J. Biol. Chem.*, **112**, 283 (1935).

[64] C. R. Harington, *Biochem. J.*, **20**, 293 (1926).

1. Syntheses of Amino Acids

There are several general methods for the laboratory synthesis of α-amino acids. The classic methods have been improved in recent times and, in addition, a number of new methods have been devised.[65, 66]

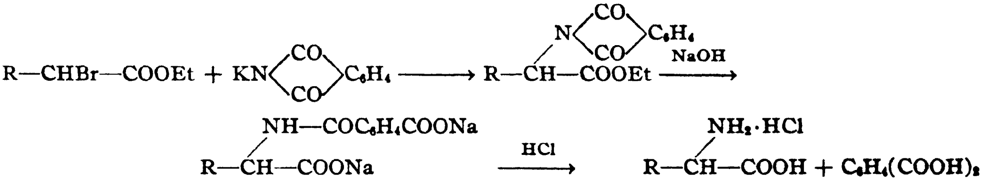
A brief outline of the general methods follows.

Halogen Acid Synthesis.—In this method, the corresponding fatty acid is halogenated in the α-position by chlorination or bromination. The halogen-substituted acid is converted into the amino acid by the action of ammonia. The steps involved in this synthesis may be simply indicated as follows:

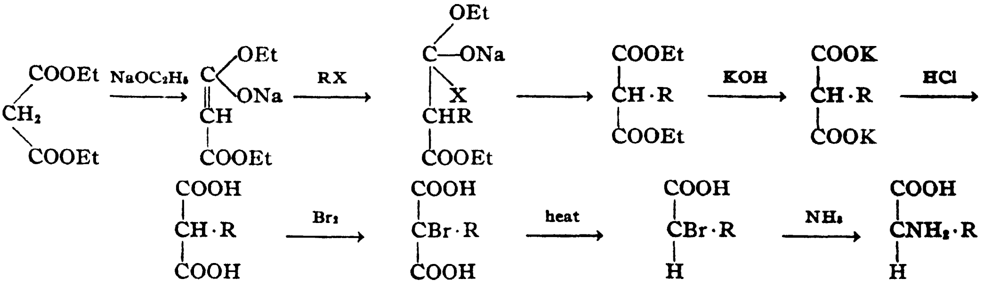
$$\mathrm{R{-}CH_2{-}COOH} \xrightarrow[\mathrm{P}]{\mathrm{Br_2}} \mathrm{R{-}CHBr{-}COOH} \xrightarrow{\mathrm{2NH_3}} \mathrm{R{-}CHNH_2{-}COOH + NH_4Br}$$

Modifications of this method, namely, the Gabriel synthesis, the malonic ester synthesis, and a combination of both of these methods, have also been used.

Gabriel Synthesis.—The Gabriel synthesis involves the interaction of potassium phthalimide and an α-bromo ester:



Malonic Ester Synthesis.—The malonic ester synthesis may be represented as follows:

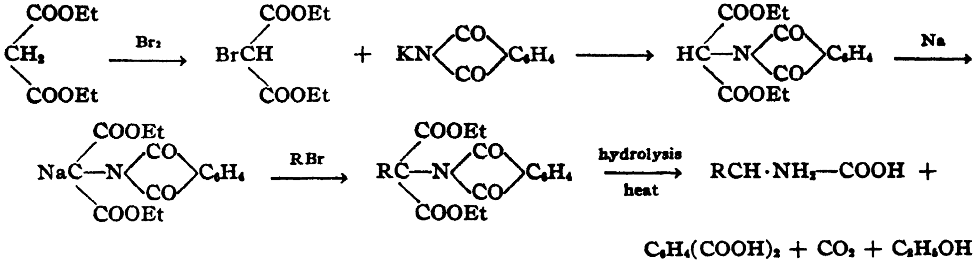


---

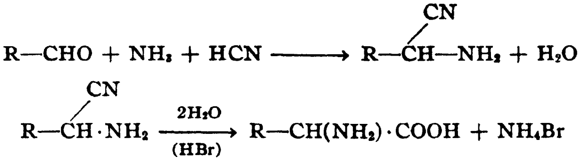
[65] H. T. Clarke, "Natural Amino Acids," in H. Gilman's *Organic Chemistry*. 2nd ed., Wiley, New York, 1943, Vol. 2, p. 1104.

[66] C. L. A. Schmidt, *The Chemistry of the Amino Acids and Proteins*. Thomas, Springfield, 1938, p. 21.

Finally, the valuable modification,[67] making use of the malonic ester and phthalimide syntheses, is illustrated by the following equations:



Cyanohydrin Synthesis.—The principle of this method, the Strecker synthesis,[68] consists of the addition of hydrogen cyanide and ammonia to aldehydes and the subsequent hydrolysis of the aminocyanohydrin with a strong acid or base.



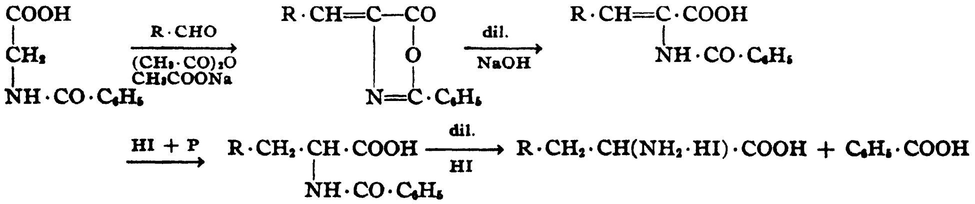
$$\text{R—CHO} + \text{NH}_3 + \text{HCN} \longrightarrow \text{R—}\overset{\text{CN}}{\text{CH}}\text{—NH}_2 + \text{H}_2\text{O}$$

$$\text{R—}\overset{\text{CN}}{\text{CH}}\cdot\text{NH}_2 \xrightarrow[\text{(HBr)}]{2\text{H}_2\text{O}} \text{R—CH(NH}_2\text{)}\cdot\text{COOH} + \text{NH}_4\text{Br}$$

The Strecker synthesis has been improved by the modified procedure of Cocker and Lapworth.[69] It is of particular value for the production of glycine, alanine, and serine.

Aldehyde Condensations.—The condensation of aromatic or heterocyclic aldehydes with hippuric acid, hydantoins, or diketopiperazine forms the basis of valuable synthetic methods for the production of phenylalanine, tyrosine, or tryptophane.

The use of hippuric acid for the synthesis of a number of amino acids (phenylalanine, leucine, and tyrosine) was first suggested by Erlenmeyer and coworkers.[70] More recently, the method has been improved and extended to the preparation of other amino acids (histidine, tryptophane, and thyroxine). The course of this synthesis (often called the azlactone method) proceeds in the following stages:



---

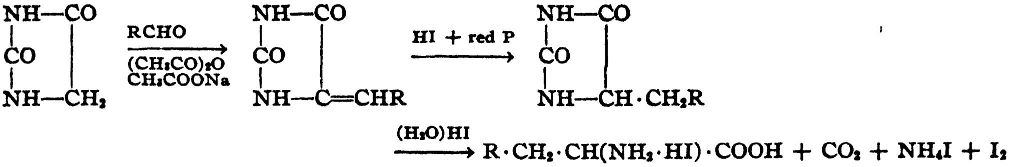
[67] S. P. L. Sørensen, *Z. physiol. Chem.*, **44**, 448 (1905).

[68] A. Strecker, *Ann.*, **75**, 27 (1850).

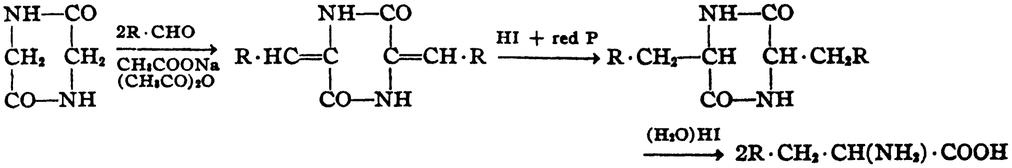
[69] W. Cocker and A. Lapworth, *J. Chem. Soc.*, **1931**, p. 1391.

[70] E. Erlenmeyer, *Ann.*, **275**, 1 (1893). E. Erlenmeyer and J. Kunlin, *ibid.*, **316**, **145** **(1901)**. E. Erlenmeyer and J. T. Halsey, *Ber.*, **30**, 2981 (1897); *Ann.*, **307**, 138 (1899).

Similar syntheses, leading to the production of phenylalanine, tyrosine, and tryptophane, have been carried out with the help of hydantoin:



In still another variation, the aromatic aldehyde is condensed with diketopiperazine (glycine anhydride):



Reduction Methods.—α-Keto acids are reduced directly or converted into the respective oximes or hydrazones and then reduced:

(*1*) $R{-}CO{\cdot}COOH + H_2 + NH_3 \xrightarrow{\text{catalyst}} R{\cdot}CH(NH_2){\cdot}COOH + H_2O$

(*2*) $R{-}CO{\cdot}COOH \xrightarrow{NH_2{\cdot}OH{\cdot}HCl} R{\cdot}C(:NOH){\cdot}COOH \xrightarrow{H_2} R{-}CH(NH_2){\cdot}COOH$

(*3*) $R{-}CO{\cdot}COOH \xrightarrow{C_6H_5{\cdot}NH{\cdot}NH_2{\cdot}HCl} R{\cdot}C(:N{\cdot}NH{\cdot}C_6H_5){\cdot}COOH \xrightarrow{2H_2} R{\cdot}CH(NH_2){\cdot}COOH + C_6H_5{\cdot}NH_2$

This method is beset by a number of technical difficulties and its use is limited.

SURVEY OF SYNTHETIC METHODS

Most of the amino acids may be synthesized by one or more of the above methods, but arginine, cystine, 3,5-diiodotyrosine, and thyroxin require special methods. Some references dealing with improved techniques of the classical methods and with special methods are the following: arginine,[71] aspartic acid,[72] cystine,[73] glutamic acid,[74] histidine,[75] lysine,[76] methionine,[77] proline,[78] thyroxine,[79] threonine,[80] tryptophane,[81]

---

[71] E. Schulze and E. Winterstein, *Z. physiol. Chem.*, **34**, 128 (1901-1902).

[72] M. S. Dunn and S. W. Fox, *J. Biol. Chem.*, **101**, 493 (1933).

[73] E. Fischer and K. Raske, *Ber.*, **41**, 893 (1908).

[74] M. S. Dunn, B. W. Smart, C. E. Redemann, and K. E. Brown, *J. Biol. Chem.*, **94**, 599 (1931-1932).

[75] F. L. Pyman, *J. Chem. Soc.*, **109**, 186 (1916).

[76] J. C. Eck and C. S. Marvel, *J. Biol. Chem.*, **106**, 387 (1934).

[77] G. Barger and T. E. Weichselbaum, *Organic Syntheses.* Vol. 14, Wiley, New York, 1934, p. 58.

[78] S. P. L. Sørensen and A. C. Andersen, *Z. physiol. Chem.*, **56**, 236 (1908).

[79] C. R. Harington and G. Barger, *Biochem. J.*, **21**, 169 (1927).

[80] H. E. Carter, *J. Biol. Chem.*, **112**, 769 (1936). M. L. Wood, R. J. Madden, and H. E. Carter, *ibid.*, **117**, 1 (1937).

[81] W. J. Boyd and W. Robson, *Biochem. J.*, **29**, 542, 546, 555, 2256 (1935).

TABLE 14

SYNTHETIC METHODS FOR THE PRODUCTION OF AMINO ACIDS

Amino acid	Principal material	Synthetic method	Theoretical yield, %
Alanine	Acetaldehyde, ammonium chloride, sodium cyanide	Cyanohydrin	50–60
	Acetaldehyde, ammonia, hydrocyanic acid	Cyanohydrin	71.8
Arginine	Cyanamide, ornithine	Schulze, Winterstein[71]	37
	Ornithuric acid, cyanamide	Sørensen, coworkers[84]	12.5
Aspartic acid	Diethyl fumarate, alcoholic ammonia	Dunn, Fox[72]	59
Cystine	Serine ester hydrochloride	Fischer, Raske[73]	25
Glutamic acid	α-Ketoglutaric acid, palladium black	Knoop, Oesterlin[85]	23
	Benzoylaminomalonic ester	Malonic ester	16
Glycine	Chloroacetic acid, concentrated ammonia	Halogen acid	55–60
Histidine	4-Chloromethylglyoxaline, ethyl chloromalonate	Malonic ester	23
	Glyoxaline formaldehyde, hippuric acid	Azlactone	11
β-Hydroxyglutamic acid	Ethyl acetone, acetone dicarboxylate, ethyl nitrite, reduction in presence of catalyst	Harington, Randall[86]	60
Hydroxyproline	α,δ-Dichlorovalerolactone	Malonic ester	..
3,5-Diiodotyrosine (iodogorgoic acid)	Tyrosine	Iodination	48 (minimum value reported)
Isoleucine	*sec*-Butyl iodide	Malonic ester	18
Leucine	Isovaleraldehyde, hydrocyanic acid, ammonia	Cyanohydrin	48
	sec-Butyl bromide	Malonic ester	37
Lysine	γ-Chlorobutyronitrile	Malonic ester	15.5
	Cyclohexanone	Eck, Marvel[76]	23
Methionine	β-Methylthiolpropionaldehyde	Cyanohydrin	3.4–6
	β-Methylthiolethyl chloride	Phthalimidomalonic ester	7–15
Norleucine	α-Bromo-*n*-caproic acid, ammonia	Halogen acid	65
Phenylalanine	Benzaldehyde, hippuric acid, hydroiodic acid, red phosphorus	Aldehyde condensation	40
	Benzoyl chloride, malonic ester, etc.	Malonic ester	50–60
	Benzaldehyde, hydantoin, etc.	Aldehyde condensation	50–60
	Benzaldehyde, diketopiperazine, etc.	Aldehyde condensation	50–60
	Benzoyl chloride, phthalimidomalonic ester	Phthalimidomalonic ester	50–60
Proline	1,2-Dicarbethoxypyrrole	Signaigo, Adkins[87]	31.8
	Cyclopentanone	Corse, Hillis	
Serine	Formic ester, hippuric ester	Erlenmeyer, Stoop[88]	48
	Ethyl acrylate through α-bromo-β-ethoxypropionate	Wood, du Vigneaud[89]	47
Threonine	Ethyl crotonate through α-bromo-β-methoxybutyric acid	Halogen acid	..
Thyroxine	Quinol monomethyl ether, 3,4,5-trinitrobenzene	Harington, Barger[79]	..
	Hydroquinone monomethyl ether, 3,4,5-trinitrobenzene	Savitzkii[90]	..
Tryptophane	Indole-3-aldehyde and hydantoin	Aldehyde condensation	17
Tyrosine	Anisaldehyde, hydantoin	Aldehyde condensation	66
	Anisyl bromide, phthalimidomalonic ester	Phthalimidomalonic ester	34
	Glycine anhydride, anisaldehyde	Aldehyde condensation	48
Valine	α-Bromoisovaleric acid, ammonia	Halogen acid	70

and tyrosine.[82] References to the original literature and critical discussions of the syntheses of all the amino acids are found in footnotes 65 and 66.

A study of the more recent methods discloses the fact that satisfactory syntheses are available for a large number of the amino acids. Many of the procedures developed in recent times are modifications of the classic methods. The important advances in technical procedures include the application of physicochemical principles, such as the mass action law to the α-halogen acid method; the simultaneous reduction and hydrolysis of unsaturated intermediate compounds with the help of phosphorus and hydriodic acid; the hydrolytic cleavage of aldehyde condensation products with concentrated hydriodic acid solution; the use of high-boiling solvents for refluxing; and the process of fractional crystallization from selected solvents.

In spite of the many important advances in technical procedures, satisfactory syntheses are lacking for arginine, cystine, hydroxyglutamic acid, hydroxyproline, histidine, thyroxin, and tryptophane. It is fortunate, indeed, that some of the amino acids, which can be synthesized with difficulty only, may be isolated readily from proteins. Quoting Plimmer[83] "The following lists show the best methods of preparing the amino acids:

By Synthesis
Glycine
Alanine
Valine
Leucine
Isoleucine
Norleucine
Serine
Phenylalanine
Aspartic acid
Hydroxyglutamic acid
Methionine

By Isolation from Proteins
Tyrosine
Cystine
Tryptophane
Histidine
Lysine
Arginine
Glutamic acid
Proline
Hydroxyproline
Methionine"

Methods which may be used for the synthesis of the various naturally occurring amino acids are indicated in Table 14.

2. Resolution of Racemic Amino Acids

With the exception of glycine, all the amino acids contained in proteins possess at least one optically active carbon atom and, provided racemization is avoided during the process of isolation, they may be obtained in the optically active form. Racemization of optically active amino acids may be brought about by refluxing with strong hydrochloric acid, by treatment with acetic anhydride which causes racemization of the acetylated amino acid to take place, and by racemization of the sodium salt of the acetyl derivative by acetic anhydride. Dilute alkali is particularly effective for the racemization of proteins and polypeptides. .

---

[82] T. Sasaki, *Ber.*, **54,** 163 (1921).

[83] B. Harrow and C. P. Sherwin, *A Textbook of Biochemistry.* Saunders, Philadelphia, 1935, p. 169.

[84] S. P. L. Sørensen, M. Hȯyrup, and A. C. Andersen, *Z. physiol. Chem.*, **76,** 44 (1911–1912).

[85] F. Knoop and H. Oesterlin, *Z. physiol. Chem.*, **148,** 294 (1925).

[86] C. R. Harington and S. S. Randall, *Biochem. J.*, **25,** 1917 (1931).

[87] F. K. Signaigo and H. Adkins, *J. Am. Chem. Soc.*, **58,** 709, 1122 (1936).

[88] E. Erlenmeyer and F. Stoop, *Ann.*, **337,** 236 (1904).

[89] J. L. Wood and V. du Vigneaud, *J. Biol. Chem.*, **134,** 413 (1940).

[90] A. Ya. Savitzkii, *Chem. Abstracts*, **30,** 1761 (1936).

The synthetic processes lead to the formation of the inactive (racemic) form. Optically active isomers often differ in physiological activity and the resolution of the externally compensated mixture is particularly important in the case of investigations dealing with the physiological activity of the optical isomers. With the exception of cystine, hydroxyproline, β-hydroxyglutamic acid, isoleucine, and threonine, two stereoisomeric forms exist for each optically active amino acid.

Resolution of racemic mixtures of amino acids has been effected by mechanical, biological, and chemical means.[91]

The optically active forms of a particular amino acid are mirror images of each other but the mechanical method of separation requires the presence of large crystals. This condition is rarely attained, and the mechanical method, therefore, has little practical value.

Biological organisms, such as yeasts, bacteria, and molds, may be used for the separation of racemic mixtures. The mixture to be separated is inoculated with the organism, and the latter is allowed to grow. During this process of growth the naturally occurring amino acid, serving as food, is completely destroyed. This method is of value only for the preparation of the unnatural variety of the amino acid and the identification of the biologically active form.

Resolution has most often been effected by means of the chemical method. Amino acids, in general, show little affinity for the optically active bases. By increasing the acidic nature of the amino acids, the formation of salts with brucine, cinchonine, quinine, and strychnine becomes feasible. Accentuation of the acidic nature may be brought about by the preparation of the acetyl, benzoyl, or formyl derivatives. The derivatives thus formed readily react with the alkaloids to form crystalline products. The difference in solubility of the salts of the *d*- and *l*-forms permits the separation of the derivatives by means of fractional crystallization from appropriate solvents. The use of *l*-methoxyacetyl chloride and *d*-camphoric acid forms the basis of more recent alternative methods.

Cystine, hydroxyproline, β-hydroxyglutamic acid, isoleucine, and threonine possess two asymmetric carbon atoms. The separation of the four stereoisomers possible in each case still awaits satisfactory solution.

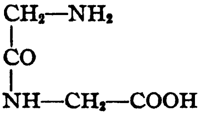
3. Synthesis of Polypeptides and Diketopiperazines

Proteins, as was previously stated, may be broken down into simpler products by the action of acid, alkali, or proteolytic enzymes. It has been found, particularly while studying the action of enzymes, that certain amino acids are liberated more readily than others. Thus, in the case of tryptic digestion, cystine, tyrosine, and tryptophane are liberated much more readily than leucine, alanine, and valine, while some amino acids, such as proline and phenylalanine, are not liberated even after long periods of digestion. This process of cleavage, therefore, leads to the formation of various mixtures of substances intermediate in molecular magnitude between proteins and amino acids. The proteins are thus reduced through various stages to proteoses, peptones, polypeptides, peptides, and on completion of the process to the "building blocks," the α-amino acids.

---

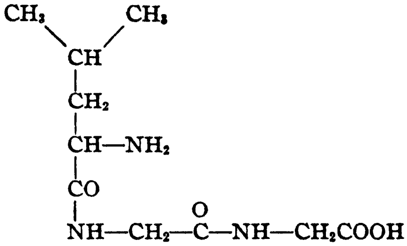
[91] H. Mahn, in Abderhalden's *Biochemisches Handlexicon.* Vol. 12, Supplement 5, Springer, Berlin, 1930.

The classic researches of Fischer and Kossel indicated that proteins were made up of amino acids. Kossel advanced the theory that amino acids were connected to each other by way of the peptide linkage, —CONH—, to yield eventually the very complex protein molecules. Fischer proceeded to synthesize relatively large molecules made up of amino acids, the latter being linked by way of this peptide linkage. Fischer thus developed methods for the synthesis of compounds which he called peptides, the simplest of which is glycylglycine. Depending on the number of amino acids in the molecule, these substances are referred to as dipeptides, tripeptides, etc.



$$\begin{array}{l}CH_2\text{—}NH_2\\ |\\ CO\\ |\\ NH\text{—}CH_2\text{—}COOH\end{array}$$

Glycylglycine



Leucylglycylglycine

Fischer in this manner synthesized a great variety of polypeptides, the most complex of which contained 18 amino acids, namely, *l*-leucyl-triglycyl-leucyl-triglycyl-leucyl-octaglycyl glycine. Abderhalden, later, prepared a polypeptide containing 19 amino acids. These high molecular weight polypeptides, although far removed in complexity from the native proteins, are in many respects similar to the natural proteoses and peptones. They give some of the color reactions which are characteristic of proteins; they are precipitated by protein precipitants, such as phosphotungstic acid and tannic acid; and they do not diffuse through some of the semipermeable membranes.

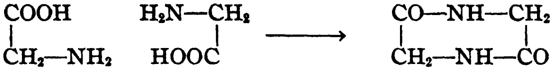
Examination of the structures of the various amino acids suggests other means by which amino acids may be joined to give rise to the more complex molecules. In addition to the α-amino and α-carboxyl groups present in all the naturally occurring amino acids, we also find other reactive groups such as the guanidine nucleus of arginine, the γ-carboxyl group of glutamic acid, the imidazole ring of histidine, the ε-amino group of lysine, and others. Researches carried out in this connection, however, point to the peptide bond as the major linkage in the case of the naturally occurring products. Thus, it has been found that the action of enzymes on protein results always in the liberation of one carboxyl group for every amino group set free:

$$\text{—CONH— + HOH} \longrightarrow \text{—COOH + —NH}_2$$

The number of free amino groups and of carboxyl groups in the native protein is relatively small. Quantitative methods for the determination of free amino nitrogen (Sørensen's formol titration and the Van Slyke method for amino nitrogen) give relatively low values. Furthermore, it has been found that alkaline hydrolysis of proteins leads to racemic mixtures of the amino acids. This fact is significant because only those amino acids are racemized whose carboxyl groups are in chemical combination, as is the case in the peptide linkage. Further evidence is found in the action of proteolytic enzymes upon native proteins, on the one hand, and upon synthetic polypeptides, on the other. Both tryptic and ereptic enzymes hydrolyze the bond occurring in the native protein as well as the linkage found in the synthetic polypeptide. Since the action of enzymes is highly specific in nature, it must be assumed that the linkage by which the amino acids are joined is the same for both substances. The problem, it must be emphasized, is not as simple as might appear from what has been said thus far. Thus pepsin hydrolyzes proteins to form a mixture of polypeptides, known as peptones. The failure of further action on the part of pepsin indicates that proteins must be more complex in structure than the polypeptides. In any case, it has been shown that the action of different proteolytic enzymes on the same protein is clearly different. Different peptidases will produce different cleavage products when acting on the same peptide molecule. This, of course, indicates that different peptidases cleave the polypeptide chain at different positions. The study of the products of hydrolysis obtained by the action of a particular peptidase upon synthetic polypeptides leads to an understanding of the molecular configuration required for the specific action. These investigations require the use of synthetic polypeptides and the need for the synthetic production of a great variety of these substances is self-evident.

A brief account of the principles involved in some of the methods for the synthesis of polypeptides is appropriate at this point.

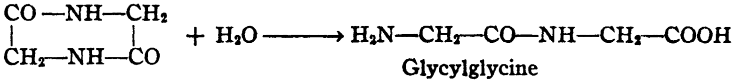
Method 1.—An aqueous solution of the ethyl ester of glycine, when permitted to stand for several days, is converted into an anhydride:



Glycine

Glycyl anhydride (diketopiperazine)

On boiling the anhydride with concentrated hydrochloric acid, Fischer and Fourneau[92] obtained the salt of the dipeptide, glycylglycine, from which they prepared the free acid by treatment with silver oxide. The equation for the hydrolysis of the anhydride is as follows:

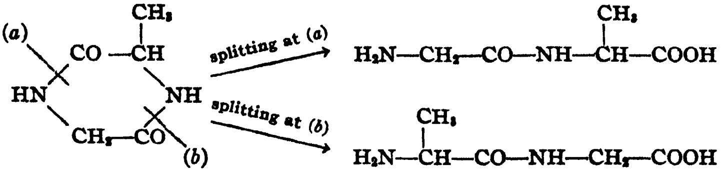


Glycylglycine

This method lends itself only to the preparation of dipeptides. The anhydride rings (diketopiperazines) formed in the first step of the synthesis show varying degrees of stability toward the action of hydrochloric acid, depending on the nature of the amino acids composing the ring. This method is not applicable to the synthesis of mixed dipeptides. The hydrolysis of a heterogeneous diketopiperazine yields two different dipeptides. Thus glycylalanine diketopiperazine gives rise to alanylglycine and glycylalanine. These peptides are extremely difficult to separate.

---

[92] E. Fischer and E. Fourneau, *Ber.*, **34**, 2868 (1901).



Method 2.—On treatment of an amino acid with a halogen-substituted acid chloride, the following reaction takes place:

$$\mathrm{Cl{-}CH_2{-}COCl + H_2N{-}CH(CH_3){\cdot}COOH \longrightarrow Cl{-}CH_2{-}CO{-}HN{-}CH(CH_3){\cdot}COOH}$$

On treating the product with ammonia, a dipeptide results:

$$\mathrm{Cl{-}CH_2{-}CO{-}HN{-}CH(CH_3){\cdot}COOH + NH_3 \longrightarrow H_2N{-}CH_2{-}CO{-}HN{-}CH(CH_3){\cdot}COOH + HCl}$$

The acid chlorides of the amino acids are very difficult to prepare. This has been accomplished in some cases, and these substances will react with an amino acid to form a dipeptide, or with a dipeptide to form a tripeptide.

$$\mathrm{CH_2(NH_2){\cdot}COCl + H_2N{-}CH_2{-}CO{-}HN{-}CH(CH_3){\cdot}COOH \longrightarrow H_2N{-}CH_2{-}CO{-}HN{-}CH_2{-}CO{-}HN{-}CH(CH_3){\cdot}COOH + HCl}$$

In general, however, by condensing different halogen-substituted acid chlorides with different amino acids various dipeptides may be produced. This method may be used for the synthesis of tripeptides, and polypeptides in general.

$$\underset{\text{Bromoisocaproyl chloride}}{\mathrm{Br{-}CH(C_4H_9){-}COCl}} + \mathrm{H_2N{-}CH_2{-}CO{-}NH{-}CH_2{-}COOH} \longrightarrow$$

$$\mathrm{Br{-}CH(C_4H_9){-}CO{-}NH{-}CH_2{-}CO{-}NH{-}CH_2{-}COOH + NH_3 \longrightarrow}$$

$$\underset{\text{Leucylglycylglycine}}{\mathrm{H_2N{-}CH(C_4H_9){-}CO{-}HN{-}CH_2{-}CO{-}NH{-}CH_2{-}COOH}} + \mathrm{HBr}$$

By continuing this procedure the chain may be lengthened.

It must be clearly understood that this method possesses a number of limitations The corresponding halogen acyl derivative for every amino acid is not available. The procedure is useful only in connection with the simple monoamino acids and basic amino acids cannot be introduced into the peptide chains by the application of this procedure. In some cases, the treatment of the halogen derivative of the peptide with ammonia results in the formation of a hydroxy peptide rather than the desired amino peptide. By the use of the correct optically active form of the acid chloride, it is possible to synthetize peptides containing the natural forms of the amino acids; the possibility of a Walden inversion during the reaction with ammonia must be taken into account when selecting the optically active acyl chloride. Finally, by the use of this method the chain may be lengthened but at one end. This procedure was first described by Fischer and Otto.[93]

Fischer applied a combination of methods 1 and 2 in his famous synthesis of the octadecapeptide mentioned above. In this connection it is important to mention that the esters of tripeptides of the simple monoamino and monocarboxylic acids condense directly to form the hexapeptides. This method, which lends itself only to the synthesis of hexapeptides from the corresponding tripeptides, was used successfully by Fischer in his syntheses of long-chain polypeptides. The principal steps of Fischer's synthesis of the octadecapeptide follows:

---

[93] E. Fischer and E. Otto, *Ber.*, 36, 2106 (1903).

(1) Diketopiperazine $\xrightarrow[\text{hydrochloric acid}]{\text{hydrolysis}}$ glycylglycine $\xrightarrow[\text{chloride}]{\text{chloroacetyl}}$ chloroacetylglycylglycine $\longrightarrow$ glycylglycylglycine (diglycylglycine) $\xrightarrow[\text{ester of glycylglycylglycine}]{\text{Condensation of}}$ glycyl-glycyl-glycyl-glycyl-glycyl-glycine (pentaglycylglycine)

(2) Glycylglycylglycine $\xrightarrow[\text{chloride}]{\alpha\text{-bromoisocaproyl}}$ α-bromoisocaproyl-glycyl-glycyl-glycine

(3) Coupling of products obtained in (1) and (2) and subsequent treatment with ammonia yields the decapeptide leucyl-glycyl-glycyl-glycyl-glycyl-glycyl-glycyl-glycyl-glycyl-glycine (leucyloctaglycylglycine)

(4) Leucyloctaglycylglycine $\xrightarrow[\text{and subsequent treatment with ammonia}]{\alpha\text{-bromoisocaproyl-glycyl-glycyl-glycine}}$ leucyl-triglycyl-leucyl-octaglycyl-glycine $\xrightarrow[\text{glycine and subsequent treatment with ammonia}]{\alpha\text{-bromoisocaproyl-glycyl-glycyl-}}$ leucyl-triglycyl-leucyl-triglycyl-leucyl-octaglycyl-glycine

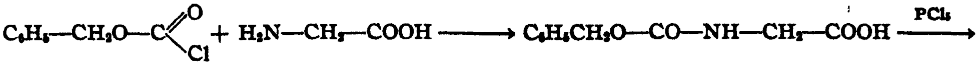
Method 3.—Thus far we have discussed methods which lend themselves to the synthesis of polypeptides consisting of the simpler monoamino and monocarboxylic acids. Since the monumental work of Fischer, methods have been devised for synthesizing polypeptides from the more complicated amino acids. By far the most direct method by which both simple and complex amino acids may be joined into polypeptide chains is the one developed by Bergmann and Zervas.[94] These investigators found that the acid chloride of an amino acid could be readily formed by protecting the amino group by means of the carbobenzoxy group (carbobenzyloxy group). The derivative containing the "blocked" amino group, on treatment with phosphorus pentachloride, yields the desired acid chloride. The resulting product in turn readily reacts with different amino acids. The carbobenzoxy group is then removed by means of catalytic reduction of hydrogen (in the presence of palladium black). It is precisely the removal of the carbobenzoxy group which marks the important advance made by this method. By means of this procedure it is possible to remove the "blocking" group without hydrolyzing the peptide linkage, and it has become possible to synthesize dipeptides containing two dicarboxylic acids, two dibasic acids, proline linked by means of the imino group, etc. The removal of the carbobenzoxy group by means of catalytic reduction is not useful in the case of cystine peptides. This removal can be accomplished by the use of phosphonium iodide[95] or by the action of sodium in liquid ammonia.[96] Harington and Mead,[95] using phosphonium iodide as the reducing agent, accomplished the brilliant synthesis of the optically active tripeptide glutamylcysteylglycine, glutathione. This is one of the most elegant applications of the modified Bergmann procedure. The various steps in the Bergmann method are illustrated by the following example:

---

[94] M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

[95] C. R. Harington and T. H. Mead, *Biochem. J.*, **29**, 1603 (1935).

[96] H. S. Loring and V. du Vigneaud, *J. Biol. Chem.*, **111**, 385 (1935).



$$C_6H_5{-}CH_2O{-}C\genfrac{}{}{0pt}{}{\nearrow O}{\searrow Cl} + H_2N{-}CH_2{-}COOH \longrightarrow C_6H_5CH_2O{-}CO{-}NH{-}CH_2{-}COOH \xrightarrow{PCl_5}$$

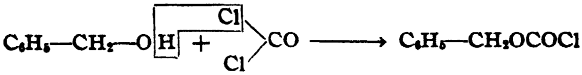
Carbobenzoxy chloride
(benzyloxycarbonyl
chloride)

$$C_6H_5{-}CH_2O{-}CO{-}NH{-}CH_2{-}COCl \xrightarrow{R\cdot CH(NH)_2COOH}$$

$$C_6H_5{-}CH_2O{-}CO{-}NH{-}CH_2{-}CO{-}NH{-}CH(R){-}COOH$$

$$\xrightarrow{2[H]} C_6H_5{-}CH_3 + CO_2 + H_2N{-}CH_2{-}CO{-}NH{-}CH(R){-}COOH$$

The preparation of benzyloxycarbonyl chloride is as follows:



Benzyl alcohol

VIII. SOME PROPERTIES OF NATURAL AMINO ACIDS[97]

The amino acids isolated from protein hydrolyzates show the characteristic reactions of both organic acids and primary amines. With the exception of proline and hydroxyproline, they all contain an amino group in α-position to the carboxyl group. Proline and hydroxyproline show marked differences in physical as well as chemical properties; this is due to the absence of the α-amino group in these molecules.

With the exception of glycine, all the natural amino acids contain one or more asymmetric carbon atoms. Therefore, the amino acids isolated from protein hydrolyzates are optically active. It appears that they have the same spatial configuration.

Amino acids are colorless, crystalline compounds and with the exception of tyrosine and cystine are easily soluble in water. The crystalline form may sometimes be used for the identification of an amino acid, *e. g.*, cystine and tyrosine. With the exception of proline, amino acids are insoluble in absolute alcohol. They are not soluble in ether or other organic solvents. Amino acids generally dissolve in aqueous alcohol; in this connection, it has been found that with increasing length of the carbon chain of the simple α-amino monocarboxylic acids, the solubility in aqueous alcohol increases and the solubility in water decreases.

1. Characteristic Reactions

As would be expected, α-amino acids undergo all the reactions of aliphatic primary amines and of organic acids. A few reactions follow.

---

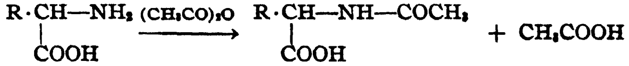
[97] See also *Handbook of Chemistry and Physics*. Hodgman and Holmes, Editors, 26th ed., Chemical Rubber, Cleveland, 1942, p. 1348.

Reaction with Nitrous Acid:

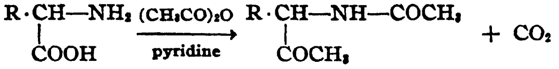
$$\mathrm{R{-}CH(NH_2){-}COOH + HNO_2 \longrightarrow R{-}CH(OH){-}COOH + N_2 + H_2O}$$

This reaction is the basis of the Van Slyke method for the determination of free amino groups in amino acids, peptides, and proteins. This reaction may also be used to differentiate between primary and secondary amines. Thus, no nitrogen is evolved when nitrous acid acts upon proline. Furthermore, the reaction may also be used to follow the progress of hydrolysis of proteins. The amino nitrogen value increases as this process of cleavage proceeds.

Reactions with Acetic Anhydride:

(a)


$$\begin{array}{l}\mathrm{R\cdot CH{-}NH_2}\\ \quad|\\ \quad\mathrm{COOH}\end{array} \xrightarrow{(\mathrm{CH_3CO})_2\mathrm{O}} \begin{array}{l}\mathrm{R\cdot CH{-}NH{-}COCH_3}\\ \quad|\\ \quad\mathrm{COOH}\end{array} + \mathrm{CH_3COOH}$$

(b)


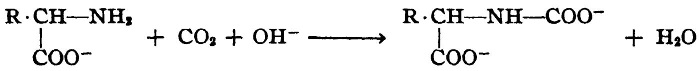
$$\begin{array}{l}\mathrm{R\cdot CH{-}NH_2}\\ \quad|\\ \quad\mathrm{COOH}\end{array} \xrightarrow[\text{pyridine}]{(\mathrm{CH_3CO})_2\mathrm{O}} \begin{array}{l}\mathrm{R\cdot CH{-}NH{-}COCH_3}\\ \quad|\\ \quad\mathrm{COCH_3}\end{array} + \mathrm{CO_2}$$

Reaction with Urea:

$$\mathrm{H_2N\cdot CO\cdot NH_2 + H_2N\cdot CHR{-}COOH \longrightarrow H_2N\cdot CO\cdot NH{-}CHR{-}COOH + NH_3}$$

A hydantoic acid

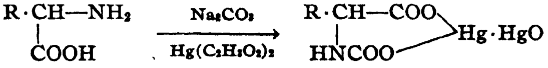
Reaction with Carbon Dioxide in an Alkaline Medium:



$$\begin{array}{l}\mathrm{R\cdot CH{-}NH_2}\\ \quad|\\ \quad\mathrm{COO^-}\end{array} + \mathrm{CO_2} + \mathrm{OH^-} \longrightarrow \begin{array}{l}\mathrm{R\cdot CH{-}NH{-}COO^-}\\ \quad|\\ \quad\mathrm{COO^-}\end{array} + \mathrm{H_2O}$$

The products thus formed are salts of carbamino acids. The calcium and barium salts of these substances show slight solubility in dilute alcohol. They decompose in boiling water to form the corresponding amino acids and carbonates of the metals. The difference in solubility of the barium salts of the carbamino acids has been used for the separation of amino acids from protein hydrolyzates.[18]

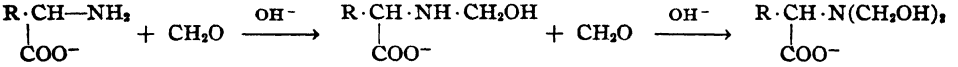
Reaction with Mercuric Acetate in the Presence of Sodium Carbonate:



$$\begin{array}{l}\mathrm{R\cdot CH{-}NH_2}\\ \quad|\\ \quad\mathrm{COOH}\end{array} \xrightarrow[\mathrm{Hg(C_2H_3O_2)_2}]{\mathrm{Na_2CO_3}} \left.\begin{array}{l}\mathrm{R\cdot CH{-}COO}\\ \quad|\\ \mathrm{HNCOO}\end{array}\right\rangle \mathrm{Hg\cdot HgO}$$

The basic mercuric salts may be almost completely precipitated upon the addition of alcohol.

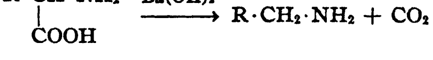
Reaction with Formaldehyde:



$$\begin{array}{l}\mathrm{R\cdot CH{-}NH_2}\\ \quad|\\ \quad\mathrm{COO^-}\end{array} + \mathrm{CH_2O} \xrightarrow{\mathrm{OH^-}} \begin{array}{l}\mathrm{R\cdot CH\cdot NH\cdot CH_2OH}\\ \quad|\\ \quad\mathrm{COO^-}\end{array} + \mathrm{CH_2O} \xrightarrow{\mathrm{OH^-}} \begin{array}{l}\mathrm{R\cdot CH\cdot N(CH_2OH)_2}\\ \quad|\\ \quad\mathrm{COO^-}\end{array}$$

Imino groups will react with but one molecule of formaldehyde. This reaction is the basis of Sørensen's formol titration used to determine the number of free carboxyl groups. Since the effect of free amino groups (basic groups) is vitiated by the treatment with formaldehyde, the titration of any free carboxyl groups is now possible.

Reaction with Barium Hydroxide:



$$\begin{array}{l}\mathrm{R\cdot CH\cdot NH_2}\\ \quad|\\ \quad\mathrm{COOH}\end{array} \xrightarrow{\mathrm{Ba(OH)_2}} \mathrm{R\cdot CH_2\cdot NH_2 + CO_2}$$

The primary amines obtained when heating amino acids with barium hydroxide are also produced during putrefactive changes.

---

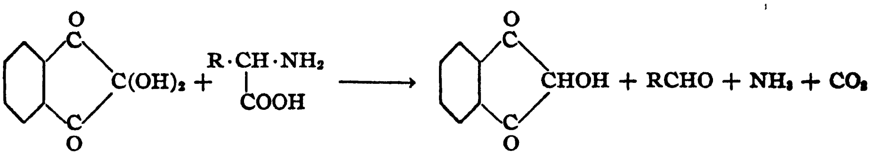
[18] S. B. Schryver and collaborators, *Biochem. J.*, **15**, 636 (1921); **18**, 1070 (1924).

Reaction with Alcohol:

$$R\cdot CH\cdot NH_2\text{—}COOH + C_2H_5OH \xrightarrow{H^+} R\cdot CH\cdot NH_2\text{—}COOC_2H_5 + H_2O$$

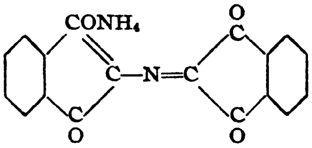
Reaction with Triketohydrindene Hydrate (*Ninhydrin Reaction*):

(*a*)



This reaction involves the dehydrogenation of the amino acid, which is converted into the corresponding aldehyde, ammonia, and carbon dioxide.

(*b*)



(Blue-colored product)

Ninhydrin and its reduction product condense with ammonia to give a blue-colored compound. Van Slyke and Dillon use this reaction for the quantitative determination of carboxyl groups in amino acids. The blue color is obtained with proteins, peptides, and amino acids which possess a free α-amino group and a free carboxyl group.

Reaction with Reinecke's Salt:

Amino acids (arginine, histidine, proline and some others) react with this salt to form characteristic crystalline derivatives. The reaction has been used for the isolation of some amino acids.

Reaction with Other Amino Acids:

The formation of diketopiperazines, dipeptides, and polypeptides is discussed on pages 134–139.

Reaction with Acids and Bases:

$$^+H_3N\text{—}CHR\text{—}COO^- + H^+ \rightleftharpoons {}^+H_3N\text{—}CHR\text{—}COOH$$

$$^+H_3N\text{—}CHR\text{—}COO^- + OH^- \rightleftharpoons H_2N\text{—}CHR\text{—}COO^- + H_2O$$

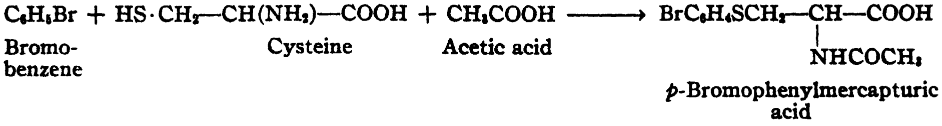
Aliphatic amino acids exist in aqueous solution mainly in the form of dipolar ions (zwitter ions). The addition of a strong acid leads to the formation of the cationic form, whereas the addition of alkali produces the anionic form of the amino acids. An excellent survey by Clarke of the general properties and reactions of natural amino acids is found in Gilman's *Organic Chemistry.*[99]

2. Amino Acids in the Process of Detoxication

Amino acids play an important role in the process of detoxication. In general the toxic substances may be rendered harmless by oxidation, reduction, internal oxidation-reduction, or by the conjugation of the toxic product with a substance found in the body of the animal to form a nontoxic product which is eventually eliminated. Amino acids, such as cysteine, glycine, glutamine derived from glutamic acid or proline, and ornithine derived from arginine, are among the substances used for the conjugation process.

---

[99] H. T. Clarke, "Natural Amino Acids," in H Gilman's *Organic Chemistry.* 2nd ed., Wiley, New York, 1943, Vol. 2, p. 1085.



$$\underset{\text{Bromobenzene}}{C_6H_5Br} + \underset{\text{Cysteine}}{HS\cdot CH_2—CH(NH_2)—COOH} + \underset{\text{Acetic acid}}{CH_3COOH} \longrightarrow \underset{p\text{-Bromophenylmercapturic acid}}{BrC_6H_4SCH_2—\underset{\substack{|\\ NHCOCH_3}}{CH}—COOH}$$

This reaction takes place in the body of the dog A similar reaction takes place when naphthalene is fed to a rabbit; the product of the reaction is naphthylmercapturic acid. Evidence has been accumulated that a similar course of detoxication may take place in the case of benzene, anthracene, and phenanthrene.

$$\underset{\text{Benzoic acid}}{C_6H_5—COOH} + \underset{\text{Glycine}}{CH_2(NH_2)—COOH} \longrightarrow \underset{\text{Hippuric acid}}{C_6H_5—CO—NH—CH_2—COOH}$$

Except in the case of the fowl, this reaction occurs in vertebrates. Using glycine, containing the nitrogen isotope N^{15}, Schoenheimer and coworkers have studied the formation of hippuric acid in rats. The presence of hippuric acid containing N^{15} indicated the direct formation of this substance from glycine.

$$\underset{\text{Phenylacetic acid}}{C_6H_5\cdot CH_2\cdot COOH} + \underset{\text{Glutamine}}{HOOC—CH(NH_2)CH_2\cdot CH_2\cdot CONH_2} \longrightarrow$$

$$\underset{\text{Phenylacetylglutamine}}{C_6H_5\cdot CH_2\cdot CO\cdot NH\cdot CH(COOH)\cdot CH_2\cdot CH_2\cdot CONH_2}$$

Whereas this reaction occurs in man, the dog and many other animals detoxicate phenylacetic acid with the help, in part at least, of glycine. Fowl, on the other hand, use ornithine for this process.

$$\underset{\text{Phenylacetic acid}}{2C_6H_5\cdot CH_2\cdot COOH} + \underset{\text{Ornithine}}{H_2N\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH(NH_2)\cdot COOH} \longrightarrow$$

$$\underset{\text{Diphenylacetylornithine}}{C_6H_5\cdot CH_2\cdot CO\cdot NH\cdot CH_2\cdot CH_2\cdot CH_2\cdot CH(COOH)\cdot NH\cdot CO\cdot CH_2C_6H_5}$$

3. Products of Intermediate Metabolism

Biochemical processes, such as intermediate metabolism and putrefaction, give rise to a large number of products derived from amino acids. A partial list of substances produced from amino acids in the animal body is indicated in Tables 15 and 16.

Amino acids (*e. g.*, alanine, arginine, aspartic acid, cystine, glycine, glutamic acid, β-hydroxyglutamic acid, hydroxyproline, norleucine, proline, and serine) which give rise to glucose in the diabetic animal (phlorizin diabetes) are glycogenic, whereas amino acids (*e. g.*, isoleucine, leucine, phenylalanine, and tyrosine) which form "acetone bodies" are said to be ketogenic.

The ammonia formed from the amino acids during the processes of intermediate metabolism is converted into urea in the case of mammals, amphibia, and many fishes. In the case of birds and reptiles, uric acid is the end product of nitrogen metabolism, whereas ammonia is the end product of this process for bacteria. According to Krebs the formation of urea from carbon dioxide and ammonia follows a mechanism outlined on page 803.

TABLE 15

POSSIBLE PRODUCTS OF THE INTERMEDIATE METABOLISM OF SOME AMINO ACIDS

Amino acid	Products
Alanine	Lactic acid, pyruvic acid, acetaldehyde, acetic acid, acetoacetic acid, glucose
Arginine	Ornithine, urea
Aspartic acid	Aminofumaric acid, ketosuccinic acid, pyruvic acid, malic acid, β-lactic acid, glucose
Cystine	Inorganic sulfates, cysteine, serine, glucose
Glycine	Glycolic acid, glycolic aldehyde, glucose
Glutamic acid	α-Ketoglutaric acid, malic acid, succinic acid, glyceric acid, glucose
Histidine	Purines, urocanic acid
Leucine	Isovaleric acid, β-hydroxybutyric acid, acetoacetic acid, acetic acid, acetone
Methionine	Homocysteine, homocystine, cysteine, cystine
Phenylalanine	Tyrosine (for other products, see tyrosine)
Proline	Glutamic acid (for other products, see glutamic acid)
Serine	α-Keto-β-hydroxypropionic aldehyde, glyceric aldehyde
Threonine	α-Amino-β-ketobutyric acid, acetic acid, glycine
Tryptophane	Indolepyruvic acid, α-aminobenzoylpyruvic acid, kynurenic acid, kynurenin
Tyrosine	Homogentisic acid (in alcaptonuria), *p*-hydroxyphenylpyruvic acid (end product in cases of tyrosinosis), 2,5-dihydroxyphenylalanine, 2,5-dihydroxyphenylpyruvic acid, 2,5-dihydroxyphenylacetic acid, acetoacetic acid

TABLE 16

AMINES PRODUCED BY THE INTESTINAL PUTREFACTION OF AMINO ACIDS

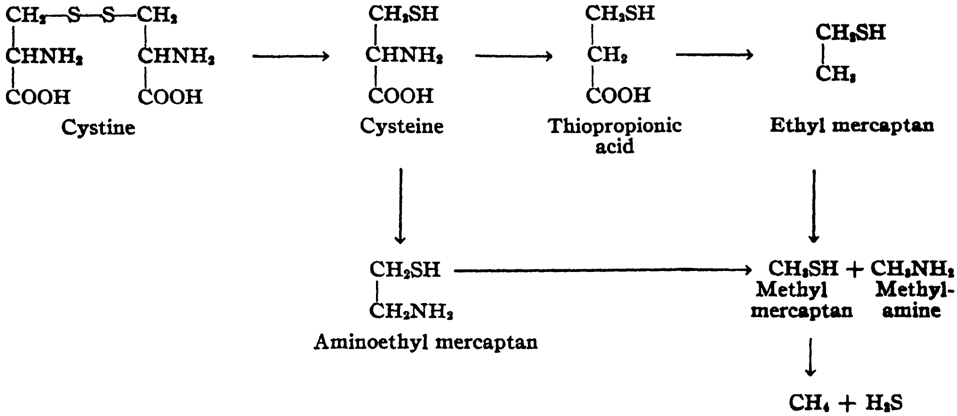
Amino acid	Products
Alanine	Ethylamine
Arginine	Ornithine, putrescine (tetramethylenediamine), agmatine (guanidobutylamine)
Glycine	Methylamine
Histidine	Histamine (β-imidazolethylamine)
Leucine	Isoamylamine
Lysine	Cadaverine (pentamethylenediamine)
Phenylalanine	β-Phenylethylamine
Tryptophane	β-Indoleëthylamine
Tyrosine	Tyramine (*p*-hydroxyphenyl-β-ethylamine)

4. Amines Produced by Intestinal Putrefaction

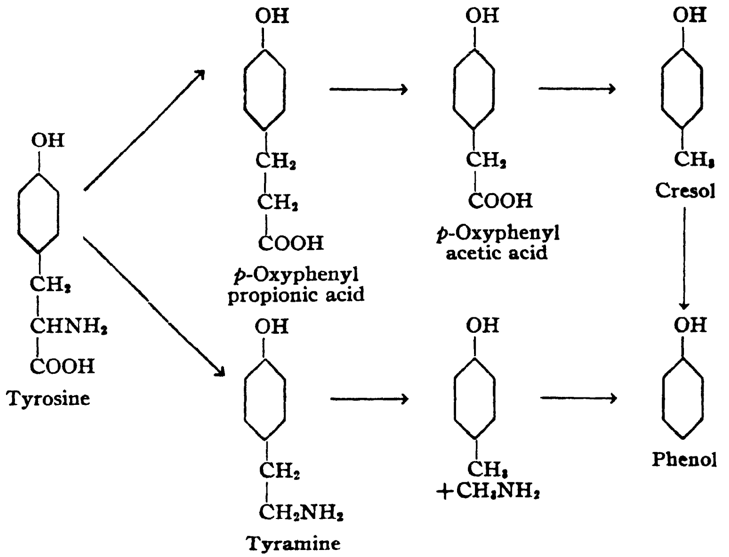
Amino acids, upon decarboxylation by means of enzymes or bacterial action, give rise to amines. These products, the result of intestinal putrefaction, may be highly toxic.

Putrescine and cadaverine are "ptomaines," substances found among the products of the putrefaction of flesh, see pages 145 and 146. Tyramine raises blood pressure and in this respect resembles adrenaline. β-Indoleëthylamine gives rise to indole and ethylamine or skatole and methylamine.

Processes of putrefaction lead to the formation of mercaptans in the case of the sulfur-containing amino acid cystine.



Other products may also result from the action of bacteria on amino acids.



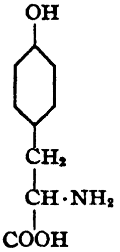
Amino acids are consequently the precursors of a great variety of biologically important substances. Block and Schoenheimer,[100] as well as other investigators, using isotopes of hydrogen and nitrogen have shown that creatine is derived from arginine, glycine, and methionine. Cystine is probably the precursor of taurine, which in turn unites with choline to form one of the bile acids. Tyrosine may be the precursor of adrenaline, the active principle of the adrenal glands. The close relationship between tyrosine, diiodotyrosine, and thyroxine, as well as the chemical nature of glutathione and insulin, is discussed elsewhere in this chapter.

---

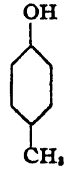
[100] R. J. Block and R. Schoenheimer, *J. Biol. Chem.*, **133**, 633; **134**, 785 (1940).

Some of the products of intermediate metabolism and intestinal putrefaction of the amino acids have been listed in order to emphasize the close relationship between the amino acids and these substances. A comparison of the structures of a few amino acids and the biologically important compounds derived from them is appropriate.

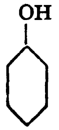
(1)



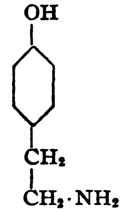
Tyrosine



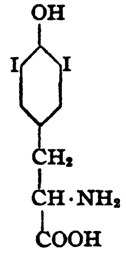
Cresol



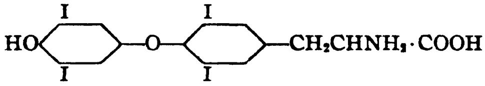
Phenol



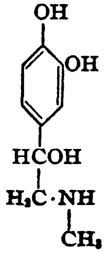
Tyramine



3,5-Diiodotyrosine

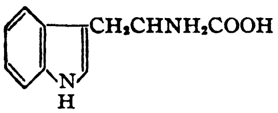


Thyroxine

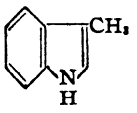


Adrenaline

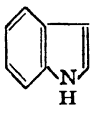
(2)



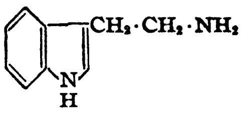
Tryptophane



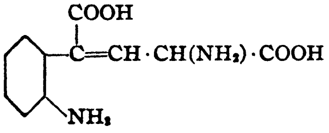
Skatole



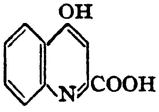
Indole



Indoleëthylamine

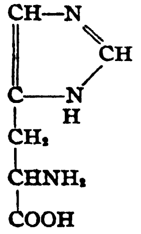


Kynurenine

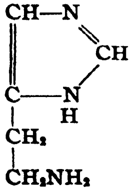


Kynurenic acid

(3)

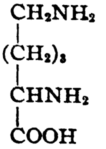


Histidine



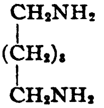
Histamine

(4)



$CH_2NH_2-(CH_2)_3-CHNH_2-COOH$

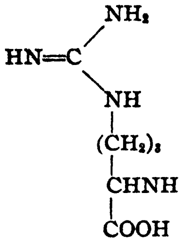
Lysine



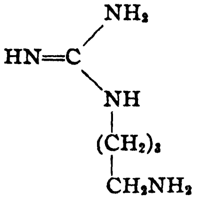
$CH_2NH_2-(CH_2)_3-CH_2NH_2$

Cadaverine

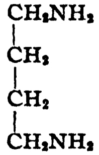
(5)



Arginine



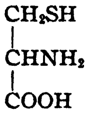
Agmatine



$CH_2NH_2{-}CH_2{-}CH_2{-}CH_2NH_2$

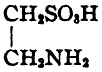
Putrescine

(6)



$CH_2SH{-}CHNH_2{-}COOH$

Cysteine



$CH_2SO_3H{-}CH_2NH_2$

Taurine

IX. SOME PROPERTIES OF PEPTIDES AND DIKETOPIPERAZINES

The isolation of dipeptides and diketopiperazines from the products of partial hydrolysis of proteins has been reported by many investigators. Thus, such dipeptides as *d*-alanylglycine from silk, glycyl-*l*-leucine and *d*-alanyl-*l*-leucine from elastin, and glycyl-*l*-tyrosine and glycyl-*d*-alanine from silk fibroin have been identified in the partially hydrolyzed product. At the same time the existence of diketopiperazines in proteins has also been reported. Fischer and Abderhalden[101] isolated from silk fibroin the diketopiperazines of glycine and *l*-tyrosine, and of glycine and *d*-alanine. In addition to the above-mentioned substances many other dipeptides and diketopiperazines have been identified in partially digested proteins.

1. Occurrence of Diketopiperazines

The existence of diketopiperazines in the protein molecules is very doubtful. The methods of hydrolysis and isolation may well lead to the formation of these ring structures. The failure of the action of proteases on diketopiperazines also strongly indicates that these substances do not exist in the protein molecules. Excellent researches carried out by Waldschmidt-Leitz and Schäffner[102] and by Greenstein[103] offer strong evidence for this view. In any case, whereas the existence of the peptide linkage in the protein molecule seems well established, the existence of diketopiperazines as structural units of the protein complex is not likely.

2. Isomeric Forms of Peptides

Peptides may occur in various isomeric forms. Two isomers may be synthesized in the case of dipeptides consisting of two different amino acids (glycylalanine and alanylglycine); six such forms are possible in the case of tripeptides containing three different amino acids (alanylglycyltyrosine, alanyltyrosylglycine, glycylalanyltyrosine, glycyltyrosylalanine, tyrosylglycylalanine, tyrosylalanylglycine). Twenty-four isomers are possible in the case of a tetrapeptide composed of four different amino acids. In general, the greater the number of different amino acids in a peptide chain, the greater is the number of possible isomers. It should be noted that the optical forms of the various amino acids are not indicated in the above examples; it is obvious, therefore, that the number of possible isomers is still greater. In view of the immense number of isomers possible in the case of the naturally occurring proteins, the synthesis of such substances presents almost insurmountable difficulties. Complete hydrolysis of proteins or polypeptide chains gives no information as to the specific order in which the constituent amino acids are linked.

---

[101] E. Fischer and E. Abderhalden, *Z. physiol. Chem.*, **46**, 52 (1905); *Ber.*, **39**, 372 (1906); **40**, 3544 (1907).

[102] E. Waldschmidt-Leitz and A. Schäffner, *Ber.*, **58**, 1356 (1925).

[103] J. P. Greenstein, *J. Biol. Chem.*, **109**, 541 (1935); **112**, 517 (1936).

3. Analysis of Peptide Chain

A useful method for the analysis of peptide chains has been developed by Bergmann and Zervas.[104] By the help of this method the constituent amino acid residues can be removed from the peptide chain one at a time. This procedure takes advantage of the Curtis reaction of the acid azides and also of the cleavage of the carbobenzoxy linkage by means of reduction. In this manner the amino acid residues removed from the peptide chain may be recognized in the form of easily identifiable aldehydes. By this method the amino acids and peptide chains are degraded from the carboxyl end of the molecule; it follows, therefore, that aminodicarboxylic acids are attacked in two positions. The degradation of peptides is best carried out by the use of the benzoylated peptides. The process as applied to a simple amino acid may be represented as follows:

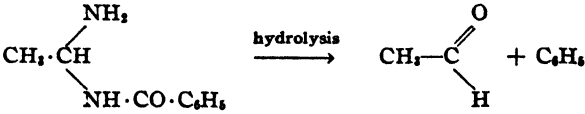
$$CH_3\cdot CH(NH_2)\text{—}COOH \xrightarrow{C_6H_5\cdot COCl} CH_3\cdot CH(NH\cdot CO\cdot C_6H_5)\text{—}COOH \xrightarrow{\text{esterify}}$$

$$CH_3\cdot CH(NH\cdot CO\cdot C_6H_5)\text{—}CO(OCH_3) \xrightarrow{NH_2\cdot NH_2} \underset{\text{Hydrazide}}{CH_3\cdot CH(NH\cdot CO\cdot C_6H_5)\text{—}CO(NH\cdot NH_2)} \xrightarrow{HNO_2}$$

$$\underset{\text{Azide}}{CH_3\cdot CH(NH\cdot CO\cdot C_6H_5)\text{—}CO(N_3)} \xrightarrow{C_6H_5.CH_2OH} \underset{\text{Benzyl urethane}}{CH_3\cdot CH(NH\cdot CO\cdot C_6H_5)\text{—}NH\cdot CO\cdot O\cdot CH_2\cdot C_6H_5}$$

$$\xrightarrow[\text{catalytic reduction}]{H_2} \underset{\text{Doubly aminated aldehyde}}{CH_3\cdot CH(NH\cdot CO\cdot C_6H_5)\text{—}NH_2} \xrightarrow{\text{hydrolysis}} \underset{\text{Aldehyde}}{CH_3CHO}$$

The final cleavage depends on the following reaction:



$$CH_3\cdot CH\begin{matrix} NH_2 \\ NH\cdot CO\cdot C_6H_5 \end{matrix} \xrightarrow{\text{hydrolysis}} CH_3\text{—}C\begin{matrix} {=}O \\ H \end{matrix} + C_6H_5CONH_2 + NH_3$$

---

[104] M. Bergmann and L. Zervas, *J. Biol. Chem.*, **113**, 341 (1936).

Thus alanine is converted into the aldehyde of one less carbon atom. In like manner the amino acid split off from a peptide chain forms an aldehyde with one less carbon atom; the latter can be identified by the usual analytical procedures. Bergmann and Zervas give the following diagram for the degradation of synthetic tetrapeptide glycyl-*l*-alanyl-*l*-leucyl-*l*-glutamic acid:

Benzoylglycylalanylleucylglutamic acid

Diester
Dihydrazide
Diazide
Dicarbobenzoxy compound

$$\Big\downarrow \text{ Hydrogenation and splitting}$$

Benzoylglycylalanylleucine amide + β-aminopropionaldehyde

Hydrazide
Azide
Carbobenzoxy compound

$$\Big\downarrow \text{ Hydrogenation and splitting}$$

Benzoylglycylalanine amide + isovaleraldehyde

Hydrazide
Azide
Carbobenzoxy compound

$$\Big\downarrow \text{ Hydrogenation and splitting}$$

Benzoylglycine amide + acetaldehyde

X. THE CHEMICAL NATURE OF SOME NORMALLY OCCURRING PHYSIOLOGICALLY ACTIVE SUBSTANCES

Two substances, thyroglobulin and insulin, are often referred to as protein hormones. The hormone action of thyroglobulin is closely connected with the iodine containing amino acid, thyroxine. The chemical nature and the specific action of the proteolytic enzymes are discussed on page 209 *et seq.*

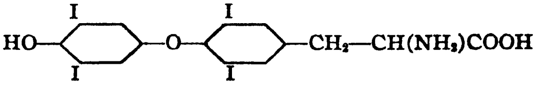
1. Thyroxine

The active principle of the globulin of the thyroid gland, thyroxine, was first isolated by Kendall of the Mayo Clinic. The process of isolation was improved by Harington[105] and its structure was determined soon after by the same investigator.[106] The synthesis was finally accomplished by Harington and Barger.[107] Another iodine derivative, 3,5-diiodotyrosine (iodogorgoic acid), which is without physiological activity, has been

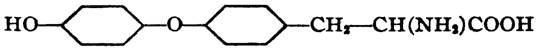
[105] C. R. Harington, *Biochem. J.*, **20**, 293 (1926).
[106] C. R. Harington, *Biochem. J.*, **20**, 293, 300 (1926).
[107] C. R. Harington and G. Barger, *Biochem. J.*, **21**, 169 (1927).

isolated from thyroglobulin by Harington and Randall, and by Foster.[108] The possibility for the existence of other iodine compounds in thyroglobulin still exists.

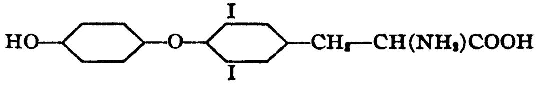
The removal of all of the iodine from thyroxine leads to the formation of thyronine, a substance without physiological activity; the loss of only two of the atoms of iodine (in the 3'- 5'-positions) leads to 3,5-diiodothyronine, which still possesses some physiological activity.



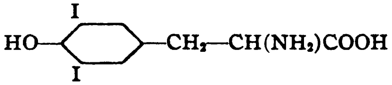
Thyroxine (physiologically active)



Thyronine (no physiological activity)



3,5-Diiodothyronine (physiologically active, but to a smaller extent than thyroxine)



3,5-Diiodotyrosine (physiologically inert)

2. Insulin

Insulin (see page 268), a hormone secreted by the ductless gland of the pancreas, is involved in the regulation of glucose metabolism. Arginine, cystine, glutamic acid, histidine, leucine, lysine, and tyrosine account for close to 90% of the products of hydrolysis obtained from insulin.[109] Crystalline insulin is a protein with a molecular weight of 41,000 (sedimentation-diffusion value). The physiological activity of this substance seems to be connected with the disulfide bond (—S—S—) of cystine and also with tyrosine. The action of alkali or pepsin on insulin brings about inactivation; in the case of the former the inactivation is due to the destruction of cystine; in the latter case it is proportional to the loss of tyrosine. Furthermore, agents which are capable of reducing the disulfide linkage render insulin physiologically inactive.

3. Glutathione

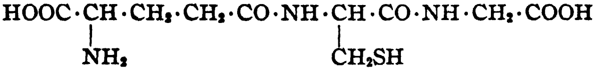
Glutathione is a tripeptide of cysteine, glycine, and glutamic acid. It was first isolated from mammalian liver, muscle, and yeast by Hopkins.[110] This substance is widely distributed in the tissues and probably plays an important role in the process of physiological oxidation. Like cystine, glutathione may be readily reduced. This, no doubt, is due to the disulfide group of the cystine component. Cysteine (the reduced form of cystine) and glutathione appear to have similar negative potentials and therefore possess similar reducing power. Some investigations indicate that glutathione may act as a coenzyme for the enzymatic reduction of methylglyoxal to lactic acid by glyoxalase. The constitution of this substance was determined by Hopkins[111] and by Kendall and coworkers.[112] Reference to the synthesis of optically active glutathione by Harington and Mead has been made on page 138.[113] The structure of the reduced form of glutathione and the changes it undergoes on oxidation and simple reduction may be represented as follows:

---

[108] C. R. Harington and S. S. Randall, *Biochem. J.*, **23**, 373 (1929). G. L. Foster, *J. Biol. Chem.*, **83**, 345 (1929).

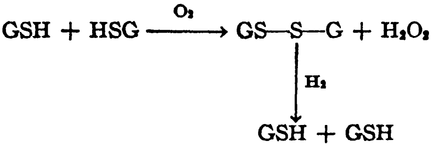
[109] H. Jensen and O. Wintersteiner, *J. Biol. Chem.*, **98**, 281 (1932).

[110] F. G. Hopkins, *Biochem. J.*, **15**, 286 (1921).



$$\underset{\displaystyle NH_2}{HOOC\cdot CH}\cdot CH_2\cdot CH_2\cdot CO\cdot NH\cdot \underset{\displaystyle CH_2SH}{CH}\cdot CO\cdot NH\cdot CH_2\cdot COOH$$

Glutamylcysteinylglycine



$$\begin{tikzcd}[column sep=small] GSH + HSG \arrow[r, "O_2"] & GS{-}S{-}G + H_2O_2 \arrow[d, "H_2"] \\ & GSH + GSH \end{tikzcd}$$

XI. THE CHEMICAL NATURE OF ANTIGENS AND ANTIBODIES

If a foreign substance such as egg albumin, be repeatedly injected into the blood stream of an animal, new substances (antibodies) appear after some time in the blood serum of the animal. The foreign protein that engenders the production of the antibodies is referred to as the antigen. The antibodies thus produced appear to react chemically with additional antigen introduced into the blood stream. These antibodies, probably modified serum globulins, show a high degree of specificity. The antibodies that have been produced by the injection of egg albumin will react only with this protein. In like manner, the albumin from horse serum when injected into a rabbit will bring about the production of antibodies which will react only with horse serum albumin. An animal which has the ability thus to remove foreign proteins is said to be immunized.

Most antigens are proteins of more or less complex structures. A few antigens are polysaccharides and perhaps lipids. The portion of the antigen which determines specificity is called *hapten.* The specific properties of antigens may be altered by chemical treatment. Thus the hapten of the synthetically produced azoproteins was shown to be the diazotized aromatic amino acids. Arsanilic acid when diazotized and coupled with horse serum forms atoxylazo horse serum. This substance when injected into the blood stream of the rabbit produces antibodies which react with atoxylazo chicken serum; the untreated sera are specific and the antisera, of course, do not cross-react. The haptens of the soluble antigens of the several types of pneumococcus are of carbohydrate nature. Many of the proteins acting as antigens in the case of infectious diseases are poorly defined. The possibility exists that lipids when in combination with proteins may act as haptens. The antigen used for the complement fixation in the Wassermann reaction is of lipid nature.

---

[111] F. G. Hopkins, *J. Biol. Chem.*, **84**, 269, 321 (1929).

[112] E. C. Kendall, B. F. McKenzie, and H. L. Mason, *J. Biol. Chem.*, **84**, 657 (1929).

[113] C. R. Harington and T. H. Mead, *Biochem. J.*, **29**, 1603 (1935).

The best experimental evidence available as to the chemical constitution of antibodies points to the protein nature of these substances. In all probability these substances are modified serum globulins. The data leading to this view are both qualitative and quantitative in nature. Molecular-weight determination by means of the sedimentation constants as obtained in the ultracentrifuge also points to the protein nature of the antibodies.

Allergy is a condition in which an individual becomes locally or wholly sensitive to a protein or several proteins. The individual may become allergic to proteins found in food, pollen, hair, etc. The anaphylaxis brought about is in part due to the chemical union of the antigen and the antibody.

The inspiring work carried out by Landsteiner, Heidelberger, and other investigators has brought about a clearer understanding of the chemical nature of antigens and antibodies. Furthermore, investigations have been carried out to find a possible explanation for the specificity of the reaction between antigen and antibody.

The substances involved in such reactions as specific precipitation, agglutination, hemolysis, and toxin-antitoxin neutralization are colloidal in nature. The mechanism of the reaction between antigen and antibody was therefore first explained by means of colloidal phenomena. These hypotheses failed to explain the high degree of specificity encountered in these reactions. More recent investigations, however, point to the chemical union between antigen and antibody. It appears that these reactions can be expressed in terms of the law of multiple proportions.

The subject dealing with the relation of proteins to immunity is treated admirably by Wells,[114] Heidelberger,[115] Landsteiner,[116] and Boyd.[116a]

XII. ANALYTICAL PROCEDURES

As has been mentioned previously, the amino acid content of different proteins may vary widely. The nutritional value of proteins depends not only on the qualitative but also on the quantitative amino acid composition. An estimation of the kind and the amount of the various amino acids present in a particular protein is of importance not only to the investigator interested in the structural problems of proteins from the point of view of the organic chemist but also to the student of nutritional problems.

The usual methods used to gain some understanding of the amino acid composition of proteins are both qualitative and quantitative in nature. Qualitative knowledge is based on the color reactions which most proteins exhibit when treated with certain reagents as well as on the many methods of isolation for the amino acids. In certain cases, *e. g.*, tyrosine, methods of isolation lead to semiquantitative or minimal results. Quantitative data are generally based on procedures dealing with titrations, colorimetry, gravimetric and volumetric estimations, and spectrophotometric analysis, *e. g.*, tyrosine, tryptophane. These methods have been devised so as to be applicable to the estimation of amino acids in protein hydrolyzates.

---

[114] H. G. Wells, *The Chemical Aspects of Immunity*. Chemical Catalog Co., New York, 1929.

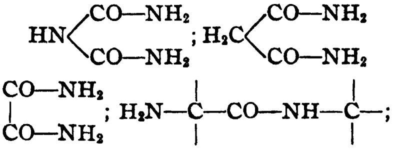
[115] M. Heidelberger, "Immunochemistry" in B. Harrow and C. P. Sherwin, *A Textbook of Biochemistry*. Saunders, Philadelphia, 1935, p. 389.

[116] K. Landsteiner, *The Specificity of Serological Reactions*. Thomas, Springfield, 1937.

[116a] W. C. Boyd, *Fundamentals of Immunology*. Interscience, New York, 1943.

TABLE 17

SOME COLOR REACTIONS OF THE PROTEINS AND AMINO ACIDS

Test	Reagents	Linkage, group, or amino acid giving the positive reaction	Color
Biuret reaction	Strong alkali, dilute copper sulfate	 $\mathrm{HN}{<}\begin{matrix}\mathrm{CO{-}NH_2}\\ \mathrm{CO{-}NH_2}\end{matrix};\ \mathrm{H_2C}{<}\begin{matrix}\mathrm{CO{-}NH_2}\\ \mathrm{CO{-}NH_2}\end{matrix}$ $\begin{matrix}\mathrm{CO{-}NH_2}\\ \vert\\ \mathrm{CO{-}NH_2}\end{matrix};\ \mathrm{H_2N{-}}\overset{\vert}{\underset{\vert}{\mathrm{C}}}\mathrm{{-}CO{-}NH{-}}\overset{\vert}{\underset{\vert}{\mathrm{C}}}{-};$ three or more amino acids joined by peptide linkage; histidine	Reddish violet to violet
Millon's reaction	Mercurous and mercuric nitrate in a solution of nitric acid	Hydroxyphenyl group; among amino acids specific for tyrosine	Brick-red
Xanthoproteic reaction	Concentrated nitric acid	Phenyl group; among amino acids tyrosine and tryptophane give test most readily	Yellow, deepens to orange on addition of alkali
Glyoxylic or Hopkins-Cole reaction	Glyoxylic acid	Indole ring; among amino acids specific for tryptophane	Violet
Ninhydrin reaction	Triketohydrindene hydrate	α-Amino acids; proline; hydroxyproline	Blue
Ehrlich's benzaldehyde reaction	*p*-Dimethylaminobenzaldehyde	Indole ring	Blue[a]
Sakaguchi reaction	α-Naphthol, sodium hypochlorite	Guanidine group; among amino acids specific for arginine	Red
Sullivan's reaction	1,2-Naphthoquinone-4-sodium sulfonate; sodium sulfite; sodium hydrosulfite; sodium cyanide	Cysteine, cystine	Red

[a] Proteins or a mixture of amino acids containing tryptophane yield a blue color when treated with Ehrlich's reagent in the presence of concentrated hydrochloric acid. Indole yields a red color. With *p*-dimethylaminobenzaldehyde in sulfuric acid, tryptophane gives a red-violet color.

Some color reactions of proteins are due to the presence of characteristic radicals or groups in the amino acids or to the type of linkages between the amino acids, while other tests are specific for a particular amino acid. A single positive or negative result does not indicate the presence or absence of protein material and several tests must be carried out in order to arrive at a definite conclusion.

1. Color Reactions of the Proteins and Amino Acids

In order for a protein hydrolyzate to give positive reactions for all of the tests listed in Table 17, it must contain three or more amino acids joined by the peptide linkage, the hydroxyphenyl group (tyrosine), the indole nucleus (tryptophane), α-amino acids, the guanidine group (arginine), and cystine or cysteine.

2. Quantitative Procedures

Sørensen's Formol Titration.—The quantitative estimation of free carboxyl groups in solutions of amino acids, peptides, and proteins is usually carried out by means of Sørensen's formol titration. The addition of formaldehyde to the test solution masks the basic nature of the free amino groups due to the formation of aldehyde ammonia derivatives. (See page 140.) The carboxyl groups present may now be determined by the titration with standard alkali, using phenolphthalein as an indicator. An interesting application of this method is its use for the determination of the purity of amino acids.[117]

Methods for the titrimetric estimation of amino acids or peptides in nonaqueous solvents (alcohol, acetone, and glacial acetic acid) have been developed by several investigators.

Van Slyke's Amino Nitrogen Method.—This is one of the best-known volumetric procedures. The principle of this method is based on the fact that nitrous acid decomposes free amino groups with the liberation of nitrogen. (See page 140.) The rate of liberation of nitrogen depends on the position of the amino group in the amino acid. Thus, the α-amino group is more readily decomposed than the ε-amino group. The reaction is carried out in a special apparatus and the nitrogen liberated is determined quantitatively. The procedure is applicable to the determination of amino nitrogen in minute quantities of proteins, peptides, or amino acids.

Sørensen's formol titration and Van Slyke's volumetric method for amino nitrogen may be used to follow the course of hydrolytic cleavage of proteins or peptides.

Methods for the Differentiation of Proteins.—Hausmann[118] devised a method for the differentiation of proteins based on the determination of nitrogen as distributed in three different groups—ammonia or amide nitrogen, basic or diamino nitrogen, and the nonbasic or monoamino nitrogen. Subsequently, a fourth group, the humin nitrogen, was added. This method, however, gives no clue as to the amino acid composition of the hydrolyzate.

Kossel and Kutscher proposed a method for the estimation of the basic amino acids arginine, histidine, and lysine. The procedure depends upon the precipitation of arginine and histidine as the respective silver salts. The difference in solubility of the silver derivatives in alkaline solution permits their separation. Lysine is precipitated by the addition of phosphotungstic acid to the filtrate from the silver salts of arginine and histidine. This method has been modified by many investigators. The most noteworthy changes have been introduced by Vickery and coworkers.[119]

Arginine, histidine, and lysine may be determined directly by precipitating histidine silver at *p*H 7.4, arginine silver between *p*H 8.5 and 14, and lysine phosphotungstate at *p*H below 1.

Van Slyke's well-known nitrogen distribution method[120] is based on the use of the Hausmann method and the Van Slyke procedure for amino nitrogen. Whereas in the Kossel and Kutscher method the basic amino acids are determined directly, the Van Slyke nitrogen distribution method permits the calculation of the amounts of arginine, histidine, lysine, and cystine from the determination of the groups which characterize these acids. Briefly, the application of this method leads to the quantitative estimation of (1) amide nitrogen; (2) humin nitrogen; (3) cystine nitrogen; (4) arginine nitrogen; (5) histidine nitrogen; (6) lysine nitrogen; (7) monoamino nitrogen; (8) nonamino nitrogen. The method fails to indicate the amino acid composition of the monoamino monocarboxylic and monoamino dicarboxylic groups.

---

[117] M. S. Dunn and A. Loshakoff, *J. Biol. Chem.*, **113**, 359 (1936).

[118] A. Hausmann, *Z. physiol. Chem.*, **27**, 95 (1899).

[119] H. B. Vickery and C. S. Leavenworth, *J. Biol. Chem.*, **72**, 403 (1927); **75**, 115 (1927); **76**, 707 (1928); **79**, 377 (1928). H. B. Vickery and R. J. Block, *J. Biol. Chem.*, **93**, 104, 113 (1931).

[120] D. D. Van Slyke, *J. Biol. Chem.*, **10**, 15 (1911).

Estimation of Individual Amino Acids.—A considerable number of methods for the estimation of individual amino acids in protein hydrolyzates have been devised. While some of the procedures proposed lead to accurate quantitative data, the trustworthiness of the results obtained by many methods is more doubtful.

Vickery, the eminent authority in the field of protein chemistry, proposes a classification of amino acids according to the degree of accuracy with which they may be determined.

TABLE 18

AMINO ACIDS CLASSIFIED ACCORDING TO DEGREE OF ACCURACY WITH WHICH THEY CAN BE DETERMINED[a]

A. Amino acids concerning which our information is little better than qualitative.

- Serine
- Threonine
- Hydroxyglutamic acid
- Valine
- Isoleucine
- Norleucine
- Thyroxine
- Diiodotyrosine

B. Amino acids for which methods of a considerable degree of probable accuracy have been proposed. These methods have been applied to very few proteins as yet.

- Glycine
- Alanine
- Leucine
- Proline
- Hydroxyproline
- Phenylalanine

C. Amino acids for which existing methods appear to give satisfactory results and which have been widely applied.

- Cystine
- Tyrosine
- Tryptophane
- Methionine
- Aspartic acid
- Glutamic acid
- Arginine
- Histidine
- Lysine

[a] H. B. Vickery, *Ann. N. Y. Acad. Sci.*, 41, 104 (1941).

The principles of a few quantitative methods are indicated below.

Alanine.—Alanine forms an insoluble derivative with dioxalatodipyridinochromiato acid (dioxopyridic acid). The weight of the derivative must be corrected for its small solubility. This procedure requires the previous removal of glycine from the protein hydrolyzate.

By another method alanine is converted to lactic acid by deamination. Lactic acid, in turn, is oxidized to acetaldehyde by means of ceric sulfate. Acetaldehyde reacting with *p*-hydroxydiphenyl yields a red-colored compound which is estimated colorimetrically. Threonine also forms acetaldehyde when subjected to this treatment.

Arginine.—Arginine yields urea on treatment with arginase. The urea may be estimated as slightly soluble dixanthydryl urea or may be determined as ammonia (urease method).

Arginine forms an impure diflavianate when treated with flavianic acid. This derivative is converted into the monoflavianate by means of boiling water and is estimated by gravimetric procedure.

Arginine, in alkaline solution, when treated with α-naphthol and sodium hypobromite yields a red-colored compound. The accuracy of this procedure is affected by many substances, such as ammonia, histidine, tyrosine, and tryptophane.

Aspartic Acid.—Aspartic acid is converted to dibromooxalacetic acid which forms a derivative with dinitrophenylhydrazine. This derivative is insoluble in water but is soluble in pyridine. The blue-colored compound which develops upon addition of sodium hydroxide is estimated colorimetrically.

Cystine.—A great number of procedures have been devised for the quantitative estimation of this amino acid.

Sullivan uses 1,2-naphthoquinone-4-sodium sulfonate for the estimation of cystine. The process is carried out in the presence of sodium sulfite, sodium hydrosulfite, and sodium cyanide. The highly reducing medium converts cystine to cysteine. The red color which develops in the presence of naphthoquinone is specific for cysteine. Cystine in the form of peptides develops a more intense and different color (magenta instead of red). Certain substances depress the color characteristic for this reaction, *e. g.*, homocystine.

By another method cystine is reduced to cysteine by means of sodium sulfite. The cysteine then reduces the phospho-18-tungstic acid to yield a blue-colored product.

Cystine may also be precipitated as cysteine cuprous mercaptide and finally estimated by the Sullivan naphthoquinone procedure (modified).

An alternate method consists in reducing cystine to cysteine by means of zinc and hydrochloric acid. The cysteine produced is titrated with potassium bromate in the presence of potassium iodide.

Glycine.—Glycine, in the absence of ammonia, is precipitated by the addition of potassium trioxalatochromiate. Under controlled conditions a constant proportion of glycine is precipitated. The weight of the derivative must be corrected for its solubility.

Glutamic Acid.—Glutamic acid is precipitated in the form of calcium glutamate. The free acid is liberated, converted into α-hydroxyglutaric acid, and finally oxidized to succinic acid by means of potassium permanganate. The silver salt of succinic acid is estimated by means of gravimetric procedure.

Histidine.—Histidine is precipitated from the protein hydrolyzate with mercuric chloride. After solution of the precipitate in sodium hydroxide, the histidine is diazotized and the resulting compound is estimated by a colorimetric procedure. The separation of tyrosine from histidine by the use of mercuric chloride is essential as tyrosine yields a red-colored compound on diazotization which would interfere with the colorimetric estimation of the histidine.

The bromination of histidine leads to the formation of a black substance which is soluble in ammonium carbonate. The blue-violet color which develops is estimated by colorimetric procedure.

Hydroxyproline.—Protein hydrolyzates, free from arginine and proline, upon treatment with Reinecke salt, yield a precipitate of hydroxyproline reineckate. The action of hypochlorite brings about the conversion of hydroxyproline to pyrrole. The latter compound reacts with isatin to give a colored product which is estimated colorimetrically.

By an alternate method, hydroxyproline is oxidized with sodium peroxide in the presence of cobalt or copper. The red chromogen produced is condensed with isatin to yield a stable red complex substance.

Lysine.—The basic amino acids are precipitated from protein hydrolyzates by the addition of phosphotungstic acid. After decomposition of the phosphotungstates, the free amino acids are subjected to the Van Slyke procedure for amino nitrogen. The α-amino nitrogen requires 5 minutes for quantitative liberation, the ϵ-amino nitrogen of lysine is liberated quantitatively in 20 minutes. The difference in the rate of reaction permits the determination of the amount of lysine present.

Methionine.—Methionine is demethylated by hydrogen iodide. The homocysteine formed is estimated by means of iodine titration. The methyl iodide formed during the process of demethylation also can be determined quantitatively.

Proline.—Proline forms an insoluble derivative with tetrathiocyanatodianilidochromiato acid (rhodanilic acid). The weight of the derivative is corrected for its solubility.

Tyrosine.—Tyrosine forms a blue color with phosphotungstic-phosphomolybdic reagent (Folin and Looney reagent). Previous to the addition of this reagent, tryptophane is removed with the help of mercuric sulfate. The Folin and Looney reagent is not specific for tyrosine.

The red color produced by the interaction of Millon's reagent and tyrosine forms the basis of another colorimetric procedure.

Tryptophane.—Acid hydrolysis destroys tryptophane. The protein is therefore subjected to enzymatic or alkaline hydrolysis. The mercuric complex of tryptophane, which is precipitated on the addition of mercuric sulfate, is decomposed and the tryptophane determined colorimetrically by the addition of sodium phosphomolybdotungstate (Folin and Denis phenol reagent). This reagent, however, is not specific for tryptophane. Other reducing substances will yield the same blue color as is obtained with tryptophane. The result should be checked by other methods.

Another colorimetric procedure is based on the fact that tryptophane reacts with mercuric chloride, mercuric sulfate, and nitrous acid to give a red color.

The Hopkins-Cole reaction for the detection of tryptophane forms the basic principle for another colorimetric method.

The reader interested in the details of experimental procedure for the estimation of the above and other amino acids must consult the original literature. A monograph by Block and Bolling[121] deals with this subject. More recent developments such as the estimation of serine, threonine, etc., will be found in the *Annual Review of Biochemistry.*[122]

The accurate establishment of the amino acid composition of the various proteins is one of the major problems of protein chemistry. The usual methods of fractionation (*e. g.*, Fischer's ester method, Dakin's butyl alcohol extraction, etc.), colorimetric estimation, and precipitation have met with varying degrees of success.

A new approach, the solubility method, leading to more accurate knowledge of the amino acid composition of proteins, has been developed by Bergmann and coworkers.[123]

The Solubility Method.—This new procedure has been developed in connection with the work on the gravimetric determination of amino acids, *e. g.*, glycine,[124] alanine,[125] etc. The incompleteness of precipitation of the derivatives of some amino acids requires the application of solubility corrections. As the solubility of the amino acid derivative is a function of the composition of the hydrolyzate from which it is precipitated, there is no sound method for the determination of the solubility correction factor. If to a protein hydrolyzate a reagent is added in quantity insufficient to precipitate the amino acid completely, the precipitate formed will contain equimolecular quantities of the amino acid and the precipitant, provided equilibrium has been reached and a binary salt is formed. The use of a known quantity of reagent permits the calculation of its concentration in the hydrolyzate after the partial precipitation of the amino acid. Assuming that molar concentrations can be substituted for ionic concentration, an equation may be set up for the solubility product of the amino acid and the precipitant. The repetition of this procedure with a different known quantity of reagent and the application of the solubility product principle permits the calculation of the molar concentration of the amino acid in the original solution. The procedure requires no knowledge of the solubility of the amino acid.

Recognizing the fact that reagents are the limiting factor in this procedure, Bergmann and Stein[126] have proposed the use of naphthalene-β-sulfonic acids for the partial precipitation of some amino acids. The salts precipitated are referred to as *nasylates*. The appreciable solubility of the nasylates is of definite advantage in the application of the solubility method. The search for satisfactory sulfonic acid reagents for the specific precipitation of amino acids is being continued in Bergmann's laboratory. The new procedure has been applied to the determination of several amino acids, such as glycine, proline, and leucine.

Bergmann's new solubility method must be regarded as a major contribution to the field of protein analysis. A more complete development and a wider application of this procedure should bring most fruitful results.

---

[121] R. J. Block and D. Bolling, *The Determination of the Amino Acids*. Burgess, Minneapolis, 1940.

[122] J. M. Luck, *Annual Review of Biochemistry*. Vols. I–XI, Annual Reviews, Inc., Stanford Univ., Calif., 1932–1942.

[123] W. H. Stein, C. Niemann, and M. Bergmann, *J. Am. Chem. Soc.*, **60**, 1703 (1938). M. Bergmann and W. H. Stein, *J. Biol. Chem.*, **128**, 217 (1939). H. R. Ing and M. Bergmann, *ibid.*, **129**, 603 (1939). S. Moore, W. H. Stein, and M. Bergmann, *Chem. Revs.*, **30**, 423 (1942).

[124] M. Bergmann and S. W. Fox, *J. Biol. Chem.*, **109**, 317 (1935). M. Bergmann and C. Niemann, *ibid.*, **122**, 577 (1937).

[125] M. Bergmann, *J. Biol. Chem.*, **122**, 569 (1938).

[126] M. Bergmann and W. H. Stein, *J. Biol. Chem.*, **129**, 609 (1939).

Another approach, leading to the better understanding of the amino acid composition of proteins, s provided by the isotope dilution method developed by Rittenberg and Foster.[137]

XIII. MOLECULAR WEIGHTS OF PROTEINS

The molecules of proteins are so large that the usual methods of determining molecular weights, *i. e.*, freezing-point method or similar procedures, cannot be applied. The depression of the freezing point, for example, is too slight to be measured with great accuracy. Furthermore, the presence of small amounts of inorganic impurities would vitiate the results obtained by methods which are applicable to the estimation of molecular weights of small molecules.

The experimental procedures which have been used for the estimation of the molecular weights of proteins may be separated into two groups. While the methods of the first group are based on stoichiometric principles, the procedures of the second group depend on the physicochemical behavior of protein solutions.

1. Determination from Chemical Composition

The minimal molecular weight of proteins may be determined by the estimation of the amount of a special element or a particular amino acid that is present in the molecule. By assuming that at least one atom of the element or one molecule of the amino acid is present in each molecule, the minimal molecular weight may be calculated by means of the following equations:

$$\text{Minimal molecular weight of protein} = \frac{\text{Atomic weight of element}}{\text{Per cent of element in protein}} \times 100$$

$$\text{Minimal molecular weight of protein} = \frac{\text{Molecular weight of amino acid}}{\text{Per cent of amino acid in protein}} \times 100$$

If the protein molecule contains n atoms of the element or n molecules of the amino acid, the value of the true molecular weight may be obtained by means of the following relationship:

$$\text{Molecular weight} = n \times \text{minimal molecular weight}$$

Whereas methods which depend on the colligative properties, such as the freezing-point depression method, cannot be used for the molecular-weight determination, these properties may be used for the evaluation of n. The accuracy of the method used for the determination of the element or the amino acid determines the trustworthiness of the minimal molecular weight value. The estimation of n, which is an integer, becomes more difficult as its value becomes larger. This fact may best be illustrated by means of a simple example. The nitrogen content of tyrosine is 7.74%; the carbon content of the same amino acid is 59.64%. (For the sake of simplicity,

---

[137] D. Rittenberg and G. L. Foster, *J. Biol. Chem.*, **133**, 737 (1940).

TABLE 19
MINIMAL AND CALCULATED MOLECULAR WEIGHTS OF EGG ALBUMIN[a]

Substance used	Observed, %	Minimal molecular weight	"True" molecular weight 36,000			"True" molecular weight 40,000		
			Assumed no. of atoms or molecules	Calculated molecular weight	Percentage of 36,000	Assumed no. of atoms or molecules	Calculated molecular weight	Percentage of 40,000
Arginine	5.66	3,075	12	36,900	102.5	13	39,970	99.9
Histidine	1.48	10,240	4	40,960	113.9	4	40,960	102.3
Lysine	4.97	2,940	12	36,240	100.7	14	41,160	102.9
Tyrosine	3.97	4,561	8	36,490	101.4	9	41,050	102.6
Tryptophane	1.18	17,300	2	34,600	96.1	2	34,600	84.5
Cystine	1.78	13,500	3	40,500	112.6	3	40,500	101.3
Methionine	5.23	2,853	13	37,090	103.0	14	39,940	99.8
Glutamic acid	13.96	1,052	34	35,760	99.3	38	39,980	99.9
Sulfur	1.616	1,983	18	35,690	99.1	20	39,660	99.1
Phosphorus	0.097	32,010	1	32,010	88.8	1	32,010	80.0
Sulfide sulfur	0.491	6,528	6	39,170	108.8	6	39,170	97.9

[a] For a more complete version of Table 19 see Vickery, "The Composition of Protein Molecules," *Ann. N. Y. Acad. Sci.*, **41**, 113 (1941).

an amino acid is chosen for this illustration.) Calculating the molecular weight by means of these values, we obtain

$$n \frac{14}{7.74} \times 100 =$$

n 181 (using the value for nitrogen)

$$n \frac{12}{59.64} \times 100 =$$

n 20 (using the value for carbon)

The accuracy with which n may be determined decreases as the error of the experimental method used for its estimation approaches the value of the minimal molecular weight. In the case of tyrosine, the error of the method used for the evaluation of n may be as large as the minimal molecular weight obtained by the use of the carbon content. Thus n might be assigned the value of 8 or 10 instead of 9. It is evident that the element or amino acid which is chosen must be present in the protein molecule in amounts small enough so that they can be used in estimating molecular weights.

Among the elements and amino acids which can be determined with a sufficient degree of accuracy and which occur in amounts small enough so they can be used in calculating molecular weights are copper, iron, sulfur, cystine, arginine, histidine, lysine, tyrosine, tryptophane, methionine, aspartic acid, and glutamic acid. In some cases sulfide sulfur, amide nitrogen, and amino nitrogen have been used.

The minimal and calculated molecular weights of egg albumin, as obtained by the estimation of different elements and amino acids, are indicated in Table 19. The assumed number of atoms or molecules (value for n) is obtained by dividing the minimal molecular weight into the value of the *true* molecular weight (36,000 by osmotic pressure measurement; 40,000 by ultracentrifuge data). Multiplication of the minimal molecular weight and the figure obtained for the assumed number of atoms or molecules gives the calculated molecular weight. An examination of the data reveals the fact that when the assumed number of atoms of the element or the molecules of the amino acid present is small, only one integer may be used for the estimation of the calculated molecular weight. On the other hand, when the number of atoms or molecules is large, more than one integer may be selected.

2. Determination from Equivalent Combining Weights

The combining capacity of proteins for certain reagents, *e. g.*, acids and bases, has also been employed for the estimation of the minimal molecular weight. It is assumed that proteins react with these reagents according to the "law of combining weights," *i. e.*, in stoichiometric proportion. The equivalent combining weight or minimal molecular weight of the protein is that amount which reacts with one equivalent weight of acid or base. The molecular weight of the protein may be calculated by multiplying the minimal molecular weight by the number of reactive groups (*i. e.*, number of free carboxyl groups if the protein was titrated with a base) present in the molecule. The relatively large number of reactive groups present in the protein molecule is difficult to estimate accurately. The base or acid-combining capacity is determined by means of electrometric titration. The titration of proteins with acidic or basic dyes forms the basic principle of another procedure for the determination of equivalent combining weights.[128]

In the case of the respiratory proteins, such as hemoglobin, oxygen or carbon monoxide combining capacity may be used to determine the minimum molecular weight. Thus it has been shown that 16,717 g. of hemoglobin combine with one mole of oxygen or carbon monoxide. This value of the minimal molecular weight of hemoglobin is in agreement with the results obtained from the content of iron, sulfide sulfur, and tryptophane.

The solubility of isoelectric proteins in acid or base has also been used as a measure of the combining capacity [129]

3. Determination from Osmotic Pressure Measurements

Of the colligative properties of solutions, only osmotic pressure measurements are accurate enough to be used for the estimation of molecular weights of proteins.

Van't Hoff found that the osmotic pressure of dilute solutions is related to the concentration of the solute (inversely proportional to the volume) and to the absolute temperature. Thus, van't Hoff derived the following relationship:

$$P_0 = CRT \text{ or } P_0V = nRT$$

where P_0 is the osmotic pressure, V is the volume of the solution, n is the moles of solute, R is the gas constant, and C is the concentration of the solute (n/V). It is obvious from this relationship that the osmotic pressure exerted by the solute is the same as if it were present as a gas and occupied the same volume as the solution. The van't Hoff relationship applies to perfect solutions. Since most solutions have the properties of perfect solutions only at great dilutions, the van't Hoff equation is fairly accurate in the case of dilute solutions, but does not apply at high concentrations of the solute. The solutions used for the experimental determination of the osmotic pressure cannot be considered as perfect solutions. Correction factors have been proposed for the volume occupied by the molecules of the solute, for the influence of ionization of the proteins, for the changes in the degree of association and dispersion of the protein molecules, for the influence of strong electrolytes, for changes of concentration of the protein, for changes due to the nature of the solvent, as well as for the hydrogen-ion activity of the solution.

---

[128] L. M. Rawlins and C L. A Schmidt, *J Biol. Chem.*, 88, 271 (1930).

[129] E. J Cohn, J. L. Hendry, and A. M. Prentiss, *J. Biol. Chem.*, 63, 721 (1925).

Outstanding contributions in this field have been made by Sørensen,[180] Adair,[181] and by Burk and Greenberg.[182]

TABLE 20

MOLECULAR WEIGHTS OF SOME PROTEINS OBTAINED BY OSMOTIC PRESSURE MEASUREMENTS

Protein	Authority	Solvent	Molecular weight
Casein	Burk and Greenberg	6.66 M urea	33,600
Edestin	Burk and Greenberg	6.66 M urea	49,500
Egg albumin	Burk and Greenberg	6.66 M urea	36,000
Hemoglobin	Adair	Buffered salt solution	68,000
Hemocyanin (*Helix pomatia*)	Adair, Adair, Roche and Roche	Buffered salt solution	1,700,000
Serum albumin (ox and sheep)	Adair and Robinson	Buffered salt solution	69,000

4. Determination by Use of the Ultracentrifuge

The most powerful tool for the direct determination of molecular weights of proteins is the ultracentrifuge. It has also been used to separate a mixture of proteins into groups of component substances according to their molecular weights. It was Svedberg who first developed this instrument and who first used it to show that proteins have definite molecular weights. Svedberg's pioneer work resulted in two methods of approach. The first depends on the measurements of the sedimentation equilibrium distributions; the second requires the estimation of the sedimentation rates. The determination of the sedimentation velocity is more rapid, but it must be followed by diffusion measurements if the molecular weight of the protein is to be determined. While the first method requires careful regulation of the speed of the ultracentrifuge and of temperature, no additional data are needed for the estimation of the molecular weight.

---

[180] S. P. L. Sørensen, *Proteins.* Fleischmann and Co., New York, 1925.

[181] G. S. Adair, *Proc. Roy. Soc. London,* **A120,** 573 (1928); **A126,** 16 (1929–1930); *J. Am. Chem. Soc.,* **51,** 696 (1929).

[182] N. F. Burk and D. M. Greenberg, *J. Biol. Chem.,* **87,** 197 (1930).

Sedimentation Equilibrium Method.—The sedimentation equilibrium method makes use of the low-speed ultracentrifuge. The proteins settle under the influence of the centrifugal field. Eventually an equilibrium is reached between the centrifugal and diffusion forces; that is, a concentration is reached near the bottom of the cell, such that the sedimentation due to the centrifugal force is balanced by the back diffusion of the protein particles. The molecular weight is then calculated by means of the following relationship:

$$M = \frac{2RT \ln C_2/C_1}{(1 - V\rho)\omega^2(X_2^2 - X_1^2)}$$

where ω = angular speed of rotation, V = partial specific volume of the solute, ρ = density of the solvent, T = absolute temperature, $R = 8.313 \times 10^7$ ergs per mole degree centigrade, and C_1 and C_2 are protein concentrations at points X_1 and X_2 from the center of rotation.

While the sedimentation equilibrium method is sound theoretically, there are certain experimental errors which should be enumerated. The possibility exists that a true equilibrium is not set up between the diffusion and centrifugal forces. The failure to maintain a constant temperature and to eliminate vibration during the process of centrifuging may affect the accuracy of the results. Since by this method equilibrium is not reached for several days, it is quite possible that some structural changes in the protein molecule may take place during the period of examination. Failure to maintain constancy in the speed of the rotor, the limiting accuracies with which the concentrations[133] and the partial specific volume can be determined, also have a bearing on the trustworthiness of the results.

Sedimentation Velocity Method.—The sedimentation velocity method has distinct advantages over the sedimentation equilibrium method. It is more rapid and can be applied more readily to the study of the homogeneity of the material under examination. Due to the relative rapidity of this procedure, the results are less likely to be affected by structural changes in the protein molecule. It cannot be used, however, when the molecular weight is smaller than 10,000.

In order to calculate the molecular weight by means of the sedimentation velocity method,[134] it is necessary to determine the specific sedimentation velocity and the diffusion constant of the protein under examination.

The specific sedimentation velocity is a constant for a particular protein in a given solvent and at a given temperature. The value of this constant, s, is calculated by means of the following equation:

$$s = \frac{dx}{dt} \cdot \frac{1}{\omega^2 x} \cdot \frac{\eta}{\eta_0} \cdot \frac{1 - V\rho_0}{1 - V\rho}$$

The molecular weight may then be determined by means of the classic Svedberg formula

$$M = \frac{sRT}{D(1 - V\rho)}$$

ω = angular speed of rotation
η = viscosity of solvent
ρ = density of solvent
η_0 = viscosity of water at 20° C.
ρ_0 = density of water at 20° C.

D = diffusion constant
dx/dt = sedimentation velocity
V = partial specific volume of solute
R = gas constant

Because of the complexity of the subject matter, the reader should refer to original articles for the detailed treatment of the theoretical and experimental aspects involved in the determination of specific volume, viscosity, diffusion constant, sedimentation velocity, and concentration gradient.[135,136]

---

[133] The concentration gradients are measured by means of photographic methods.

[134] The high-speed ultracentrifuge is used to determine the specific sedimentation velocity.

[135] While the cell containing the solution under examination is still being rotated at known speed the concentration gradient from the center to the outside edge is determined by photographic procedure.

[136] D. A. MacInnes, W. J. Archibald, J. W. Beans, W. B. Bridgman, A. Rothen, and J. W. Williams, *Ann. N. Y. Acad. Sci.*, **43**, 173 (1942). E. G. Pickels, *Chem. Revs.*, **30**, 341 (1942). J. L. Oncley, *Ann. N. Y. Acad. Sci.*, **41**, 121 (1941). T. Svedberg and K. O. Pedersen, *The Ultracentrifuge*. Oxford Univ. Press, New York, 1940. E. O. Kraemer, *J. Franklin Inst.*, **229**, 393 (1940). H. Stevenson, *Kolloid-Z.*, **87**, 181 (1939).

A study of the practical applications of the ultracentrifuge reveals that sedimentation equilibrium and sedimentation velocity procedures may be used for the estimation of the weight of the protein particles in solution, for the determination of their size and shape,[137] and for the study of the stability of protein solutions under various experimental conditions. Since it is possible to determine whether particles of varying size and weight are present in a solution of a particular protein, it also becomes possible to follow changes in particle size and particle weight which are due to variation of solvent; protein, hydrogen-ion, and salt concentration; etc.

Table 21

Molecular Weights of Some Proteins as Determined by the Ultracentrifuge[a]

Protein	M_s[b]	M_e
Erythrocruorin (*lampetra*)	17,100	19,100
Gliadin	27,500	27,000
Hemoglobin (horse)	68,000	68,000
Hemoglobin (man)	63,000	....
Hordein	27,500	....
Insulin	41,000	35,000
Lactalbumin	17,400	....
Lactoglobulin	41,500	38,000
Myoglobin	16,900	17,500
Ovalbumin	44,000	40,500
Pepsin	35,500	39,000
Serum albumin (horse)	70,000	68,000
Zein	40,000	....

[a] The existence of proteins with extraordinarily high molecular weights has been reported (*e. g*., hemocyanin from helix blood, 6,700,000; bushy stunt virus, 7,600,000; virus of the tobacco mosaic disease, 17,000,000). Because of their high molecular weight, these macromolecules may be separated from associated impurities by means of the ultracentrifuge. The physiologically active constituent of some viruses appears to be protein material of high molecular weight.

[b] M_s represents the molecular weight arrived at from the sedimentation and diffusion constants, and M_e that from equilibrium measurements.

Early investigations of Svedberg seemed to indicate that molecular weights of proteins are multiples of 34,500. Due to further molecular weight determinations, the unit was changed to 17,600. An examination of the molecular weights listed in Table 21 reveals many discrepancies; the existence of this unit, therefore, becomes most doubtful.

The molecular weights listed in Table 21 have been selected from the values reported by Pedersen.[137a]

The theories dealing with the application of the ultracentrifuge to the molecular weight determination of high molecular weight substances appear to be well established. The sources of error are being eliminated and the scope of application ever widened. The application of the ultracentrifuge to the solution of biochemical problems must be regarded as a major contribution to the field of science.

---

[137] Ultracentrifuge studies indicate that the shape of the protein molecule is approximated by an ellipsoid of revolution. The protein molecules therefore are either oblong or oblate. Experimental observations at present do not permit the differentiation between the two shapes.

[137a] K. O. Pedersen in T. Svedberg and K. O. Pedersen, *The Ultracentrifuge*. Oxford Univ Press, New York, 1940, p. 406.

XIV. STRUCTURE OF PROTEINS

Proteins possess molecular weights ranging from several thousand to several million and exhibit wide variations in physical and chemical properties. Hypotheses concerning the structure of proteins must account for both these types of properties: a hypothesis based on chemical evidence must corroborate the observations of the physical chemist and the physicist. Three different avenues (organic, physicochemical, and physical methods) of approach have been followed in an effort to gain some insight into the structure of the vastly complex protein molecules.

1. Peptide Theory

The peptide theory is based on the pioneer work of Hofmeister[138] and of Fischer.[139] According to this theory proteins are composed of chains of amino acids which are linked to each other by way of the peptide linkage. This theory is supported by such experimental evidence as: (*a*) native proteins contain a small amount of amino nitrogen; (*b*) the biuret reaction is given by peptides and proteins; (*c*) the peptide linkage is encountered in other naturally occurring substances, *e. g.*, hippuric acid; (*d*) synthetic peptides are acted upon by enzymes; (*e*) hydrolysis of proteins is accompanied by the liberation of equivalent amounts of carboxyl and amino groups; (*f*) polypeptides are present among the products of partial hydrolysis of proteins.

Although the existence of the peptide linkage in the protein molecule has been firmly established, it does not satisfactorily explain all the properties of proteins *e. g.*, denaturation of proteins. The theory that protein molecules are not simply polypeptides is supported by the fact that pepsin will initiate protein hydrolysis but fails to act upon polypeptides.

2. Reversibly Dissociable Component Systems

Sørensen[140] regards proteins as reversibly dissociable component systems. This view is based on the fact that the solubility of some purified proteins in dilute salt solutions varies with the amount of solid phase. Application of Gibb's phase rule indicates the presence of more than one substance. Sørensen assumes that some proteins which have been regarded as chemical individuals in reality give rise to a system of reversible components, *i. e.*, if $A_xB_yC_z$ represents the formula of the protein, then A, B, C, etc., indicate the components, namely, polypeptides, and x, y, z, etc., represent the number of the respective components in the whole system. It is further assumed that the atoms constituting the components are held together by primary valences, while the various components are reversibly interlinked by residual valences.

3. Distribution of Amino Acids in Peptide Chains

Investigations of Bergmann and Bergmann and Niemann indicate that particular amino acids recur *periodically* in the polypeptide chains making up the protein molecule. This observation becomes significant if it is recalled that the properties of proteins depend mainly on the number, kind, and order in which the residues are linked to one another.

---

[138] F. Hofmeister, *Ergeb. Physiol.*, **1**, 759 (1902).

[139] E. Fischer, *Ber.*, **35**, 1095 (1902).

[140] S. P. L. Sørensen, *Compt. rend. trav. lab. Carlsberg*, **18**, No. 5, 1 (1930).

The quantitative analysis of protein hydrolyzates permits the estimation of the relative number of residues in the protein. Thus, the hydrolyzates obtained from the hemoglobins of cattle, dog, and horse contain three times as much lysine as arginine. Glycine accounts for one-third, proline one-sixth, and hydroxyproline one-ninth of the amino acids present in gelatin. It is possible to estimate the ratio of the number of moles of a particular amino acid to the total number of moles of all the amino acids present in the hydrolyzate; that is, the smaller the ratio the more frequently does the amino acid occur in the molecule. The reciprocal of the ratio is the measure of the frequency with which the particular amino acid recurs in the polypeptide chain.

Investigations dealing with the frequency with which amino acids recur in different protein molecules have been carried out, particularly by Bergmann and Bergmann and Niemann.[141] These investigators found that in the case of cattle hemoglobin, for example, the frequency of occurrence of lysine, histidine, aspartic acid, glutamic acid, tyrosine, proline, arginine, and cystine is 16, 18, 18, 36, 48, 48, 48, and 192, respectively. Thus, every lysine residue is separated by 15 other amino acid residues, every histidine or aspartic acid residue by 17 other residues, etc. If we assume the hemoglobin molecule to be a continuous polypeptide chain in which the amino acids recur with regular periodicity, then the chain must contain at least 192 links. But such a chain would not accommodate a whole number of histidine, aspartic acid, and glutamic acid residues; a polypeptide chain with 576 (3 × 192) links is required in order to accommodate a whole number of residues of all the amino acids enumerated. Since the average molecular weight of all the amino acid residues is 115.5, the molecular weight of the cattle hemoglobin is 576 × 115.5 = 66,528 (reported as 66,500). This value is raised to 69,000, provided the weights of the prosthetic group and iron are added. This result is in excellent agreement with that obtained by the more direct osmotic pressure and ultracentrifuge methods.

Bergmann and Niemann propose the following hypothesis: "In every protein each amino acid residue is distributed throughout the entire peptide chain at constant intervals; *i. e.*, each amino acid residue recurs with a characteristic whole number frequency." Polypeptide chains therefore appear to consist of amino acids which recur with regular periodicity.

4. Diketopiperazine Complexes

Abderhalden[142] noticed that diketopiperazine rings are present in protein hydrolyzates. He therefore suggested that proteins are composed of diketopiperazine complexes held together by means of secondary valences. Waldschmidt-Leitz[143] has shown that diketopiperazine rings are not digested by enzymes; therefore, instead of existing as such in the protein molecule they are probably formed from amino acids during the process of hydrolysis.

---

[141] M. Bergmann, *Harvey Lectures*. Vol. 31, Williams & Wilkins, Baltimore, 1936, p. 37. M. Bergmann and C. Niemann, *J. Biol. Chem.*, **118**, 301 (1937); **122**, 577 (1938).

[142] E. Abderhalden, *Naturwissenschaften*, **12**, 716 (1924).

[143] E. Waldschmidt-Leitz and M. Görtner, *Z. physiol. Chem.*, **244**, 221 (1936).

5. Pyrrole Hypothesis

According to the pyrrole hypothesis as advanced by Troensgaard,[144] proteins are formed by the condensation of pyrrole rings. The nature of various nitrogen compounds found in the hydrolyzates of protein derivatives and the production of pyrrole derivatives on dry distillation of proteins has been offered as experimental evidence in support of the theory. Proteins give a positive reaction for pyrrole with pine shaving. This hypothesis has been refuted by Wrede.[145]

6. Protamin Nucleus Hypothesis

According to Kossel's protamin nucleus hypothesis,[146] proteins are built upon a central basic polypeptide nucleus rich in arginine. While this hypothesis has not been widely accepted, Block[147] stresses the directive influence of histidine, lysine, and arginine in the synthesis of tissue proteins; that is, the structure of some proteins appears to be dependent on the relative amount of the basic amino acid content. It is possible to classify tissue proteins in accordance with their content of arginine, histidine, and lysine.

7. X-Ray Diffraction Methods

While the ultracentrifuge is a most valuable tool for the determination of molecular weights of proteins, the x-ray has become equally important in studies dealing with protein structure. The brilliant investigations of Bernal, Crowfoot, Fankuchen, and others on crystalline proteins and Astbury's researches on fibrous proteins have yielded much information concerning the stereochemical configuration of these substances. Whereas x-ray data alone cannot lead to a complete understanding of the structure of proteins, the correlation of the results obtained by organic, physicochemical, and physical procedures has led to sounder theories of protein structure.

The crystal structure analysis by means of x-ray diffraction methods as applied to proteins is too complex to be discussed in this treatment. Introductory discussions dealing with this subject matter are found in papers by Warren[148] and Fankuchen.[149]

Applications of x-ray methods indicate that fibrous proteins contain polypeptide chains. Whereas these chains appear to be fully extended in the case of silk fibroin, wool appears to contain folded polypeptide chains.[150] Furthermore, denatured crystalline proteins also appear to contain polypeptide chains.[151]

Studies, particularly in the case of silk fibroin, indicate that polypeptide chains lie parallel or spirally inclined at a constant angle to the fiber axis. It is also assumed that the long-chain polypeptides aggregate into submicroscopic bundles (or micelles) which are held together by means of covalent linkages.

---

[144] N. Troensgaard, *Z. physiol. Chem.*, **112**, 86 (1921)

[145] F. Wrede, *Z. physiol. Chem.*, **206**, 146 (1932).

[146] A. Kossel, *Z. physiol. Chem.*, **44**, 347 (1905).

[147] R. J. Block, D. C. Darrow, and M. K. Carey, *J. Biol. Chem.*, **104**, 347 (1934). R. J. Block, *ibid.*, **105**, 445 (1934).

[148] B. E. Warren, *Ann. N. Y. Acad. Sci.*, **41**, 151 (1941).

[149] I. Fankuchen, *Ann. N. Y. Acad. Sci.*, **41**, 157 (1941).

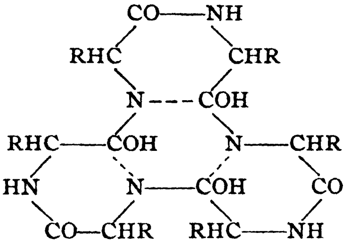
[150] W. T. Astbury, *J. Textile Inst.*, **27**, 281 (1936); *Science Progress*, No. 133 (July, 1939).

[151] W. T. Astbury, *Nature*, **140**, 968 (1937).

The work of Astbury[152] and Astbury and Woods[153] indicates that proteins, such as the keratin of hair, may undergo reversible intramolecular rearrangement. Hair, when moistened, may be stretched. The process is reversible and is accompanied by changes in the x-ray diffraction patterns. According to Astbury, α-keratin, the normal form of keratin, contains polypeptide chains in the form of hexagonal rings, while β-keratin, the denatured, longer modification, is composed of fully extended polypeptide chains. More recent investigations indicate that crystals immersed in solutions give better x-ray photographs than dry crystals. This is interpreted as being due to disorientation of the molecules in the dry crystal and to a slight rearrangement within the molecules. The researches of Bernal,[154] Astbury, Dickinson, and Bailey,[155] and Pauling and Niemann[156] show that the change from a crystalline protein to its denatured form does not involve any major rearrangement of the constituent atoms.

8. Cyclol Theory

According to the cyclol theory proposed by Wrinch, proteins are composed of closed polypeptide chains. These chains, it is assumed, are folded in hexagonal arrangements. The grouping of six amino acid residues in the form of diazine rings constituting the cyclol 6 molecule may be illustrated as follows:



While the cyclol theory explains satisfactorily many of the properties of proteins, it cannot be regarded as firmly established at present. The general acceptance of this theory requires further chemical and physical experimental support. For a detailed discussion, the reader must refer to the original papers.[157]

For recent developments in the field of protein structure see Section XVII of this chapter.

XV. CONJUGATED PROTEINS

As has been previously stated, conjugated proteins are composed of protein combined with some nonprotein substance or substances. It is the *prosthetic group* which characterizes these complexes. The conjugated proteins, among which are compounds of great biochemical importance, may be extracted from biological materials by special procedures. Depending on the nature of the prosthetic groups, these substances are usually classified as nucleoproteins, glycoproteins (glucoproteins), phosphoproteins, chromoproteins, and lecithoproteins.

---

[152] W. T. Astbury, *J. Soc. Chem. Ind.*, **49**, 441 (1930); *Nature*, **137**, 803 (1936).

[153] W. T. Astbury and H. J. Woods, *Trans. Roy. Soc. London*, **A232**, 333 (1933).

[154] J. D. Bernal, *Nature*, **143**, 663 (1939).

[155] W. T. Astbury, S. Dickinson, and K. Bailey, *Biochem. J.*, **29**, 2351 (1935).

[156] L. Pauling and C. Niemann, *J. Am. Chem. Soc.*, **61**, 1860 (1939).

[157] D. N. Wrinch, *Nature*, **137**, 411 (1936); **138**, 241, 651, 758 (1936); *Proc. Roy. Soc. London*, **A160**, 59 (1937); *Trans. Faraday Soc.*, **33**, 1368 (1937).

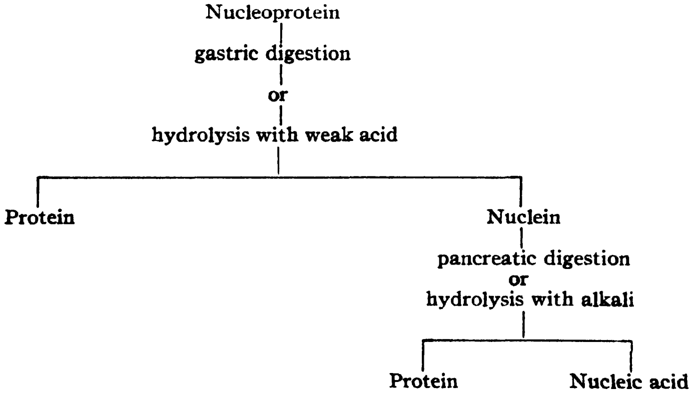
The reader must refer to the original literature and reviews for a more complete treatment of this phase of protein chemistry.

1. Nucleoproteins

Nucleoproteins are found regularly in the nuclei of all plant and animal cells. Glandular tissues, such as those derived from the pancreas and spleen, are good sources of nucleoproteins.

Nucleoproteins may be extracted from tissue by means of dilute alkali or by boiling water. Depending on the mode of extraction, nucleoproteins derived from the same source may vary in composition. In some instances at least, they may be regarded as mixtures of protein and nucleic acid.

If we assume the members of this class of conjugated proteins to consist of a relatively loose combination of basic protein and a tetranucleotide (a nucleic acid), the liberation of nucleic acid may be represented as follows:

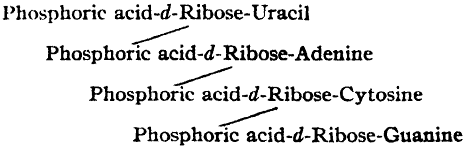


Upon vigorous hydrolysis, the nucleic acids may be split into (*1*) purine bases, (*2*) pyrimidine bases, (*3*) a carbohydrate, and (*4*) phosphoric acid. A study of the products of hydrolysis reveals two groups of nucleic acids. Yeast nucleic acid is a typical member of one group, while thymus nucleic acid belongs to the other group.

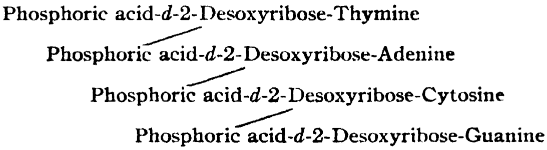
$$\text{Yeast Nucleic Acid} \xrightarrow[\text{hydrolysis}]{\text{complete}} \begin{cases} \text{1. Phosphoric acid} \\ \text{2. } d\text{-Ribose (Carbohydrate)} \\ \text{3. Adenine (Purine)} \\ \text{4. Guanine (Purine)} \\ \text{5. Cytosine (Pyrimidine)} \\ \text{6. Uracil (Pyrimidine)} \end{cases}$$

$$\text{Thymus Nucleic Acid} \xrightarrow[\text{hydrolysis}]{\text{complete}} \left\{\begin{array}{l} \text{1. Phosphoric acid} \\ \text{2. } d\text{-2-Desoxyribose (Carbohydrate)} \\ \text{3. Adenine (Purine)} \\ \text{4. Guanine (Purine)} \\ \text{5. Cytosine (Pyrimidine)} \\ \text{6. Thymine (Pyrimidine)} \end{array}\right.$$

Levene proposed the following structures for these nucleic acids:


Phosphoric acid-*d*-Ribose-Uracil
Phosphoric acid-*d*-Ribose-Adenine
Phosphoric acid-*d*-Ribose-Cytosine
Phosphoric acid-*d*-Ribose-Guanine

Yeast nucleic acid

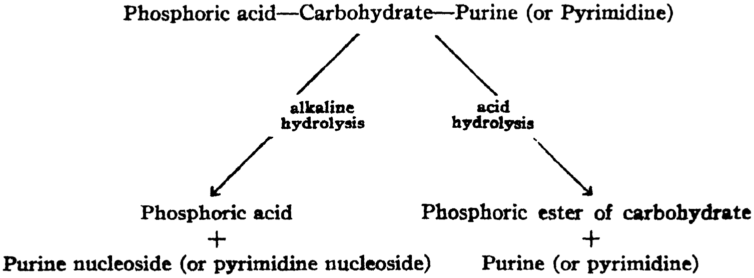

Phosphoric acid-*d*-2-Desoxyribose-Thymine
Phosphoric acid-*d*-2-Desoxyribose-Adenine
Phosphoric acid-*d*-2-Desoxyribose-Cytosine
Phosphoric acid-*d*-2-Desoxyribose-Guanine

Thymus nucleic acid

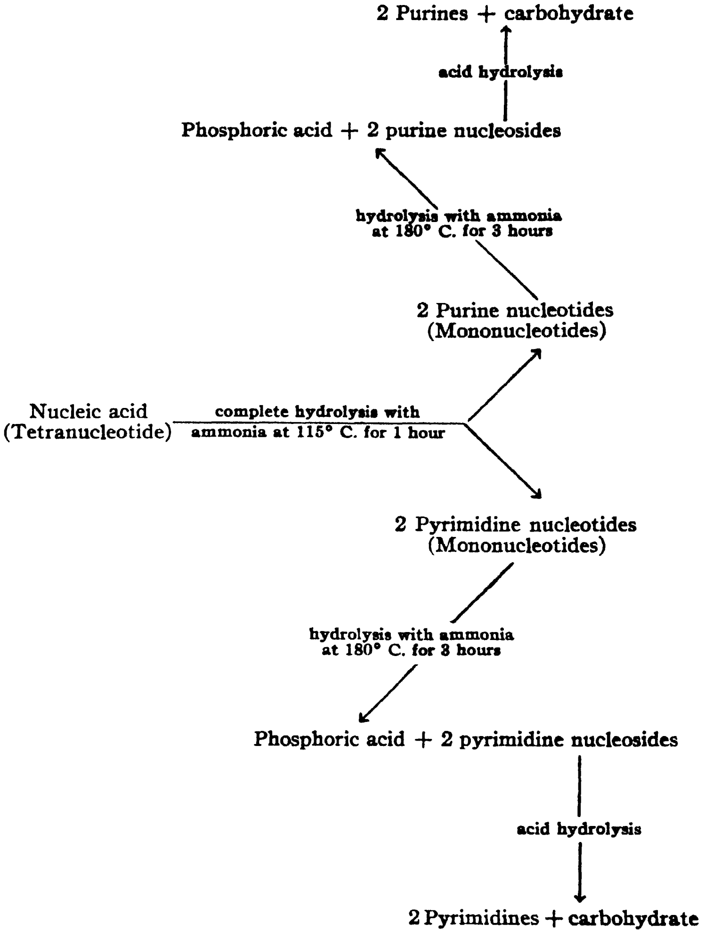
It is apparent that the two classes of nucleic acids differ in the sugar component and in one of the pyrimidines. It had been generally accepted that yeast nucleic acid was always present in the nuclei of plant cells, while thymus nucleic acid was always found in the nuclei of animal cells. More recent investigations indicate animal nucleic acid (thymus nucleic acid) to be a constituent of all plant and animal nuclei. Furthermore, plant nucleic acid (yeast nucleic acid) apparently is a constituent of cytoplasm rather than of the nucleus.

Nucleic acids may be decomposed by the action of enzymes, *i. e.*, polynucleotidases, nucleotidases, nucleosidases.

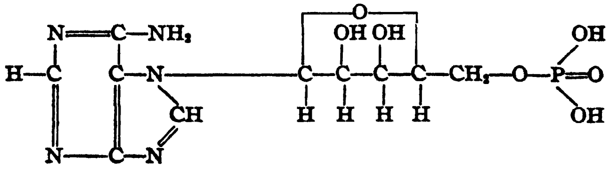
Nucleic acids upon careful hydrolysis with ammonia give rise to four mononucleotides. While the latter substances, when subjected to further alkaline hydrolysis, will give rise to nucleosides and phosphoric acid, acid hydrolysis splits off the purine or pyrimidine, leaving the phosphoric ester of the carbohydrate.


Phosphoric acid—Carbohydrate—Purine (or Pyrimidine)
alkaline hydrolysis
acid hydrolysis
Phosphoric acid
+
Purine nucleoside (or pyrimidine nucleoside)
Phosphoric ester of carbohydrate
+
Purine (or pyrimidine)

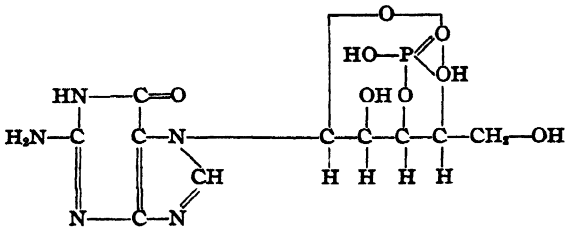
The decomposition of nucleic acids may be indicated as follows:


2 Purines + carbohydrate
acid hydrolysis
Phosphoric acid + 2 purine nucleosides
hydrolysis with ammonia at 180° C. for 3 hours
2 Purine nucleotides (Mononucleotides)
Nucleic acid (Tetranucleotide)
complete hydrolysis with ammonia at 115° C. for 1 hour
2 Pyrimidine nucleotides (Mononucleotides)
hydrolysis with ammonia at 180° C. for 3 hours
Phosphoric acid + 2 pyrimidine nucleosides
acid hydrolysis
2 Pyrimidines + carbohydrate

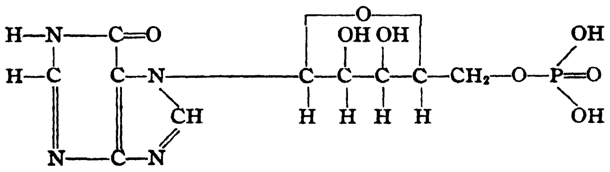
A number of mononucleotides have been isolated from biological materials. Thus, brain and heart muscle are sources for adenylic acid; liver, pancreas, and spleen for guanylic acid; and beef extract for inosinic acid. The following structures have been assigned to these mononucleotides:



Adenylic acid

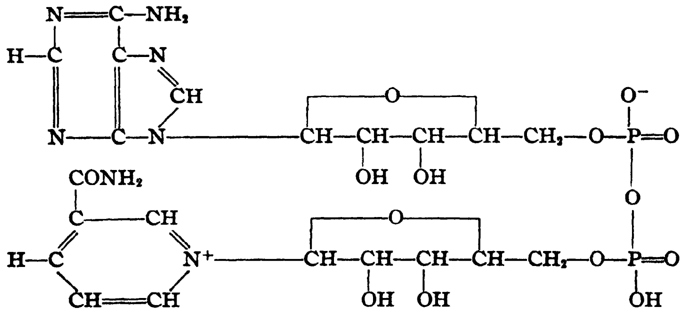


Guanylic acid



Inosinic acid

A number of coenzymes appear to be nucleotides. Thus, codehydrogenase I and codehydrogenase II (Warburg's coferment) belong to this class of substances. On the basis of chemical evidence, the following structure has been assigned to codehydrogenase.[158]

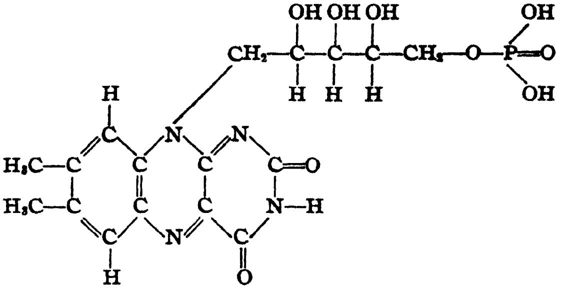


Codehydrogenase I (diphosphopyridine nucleotide)

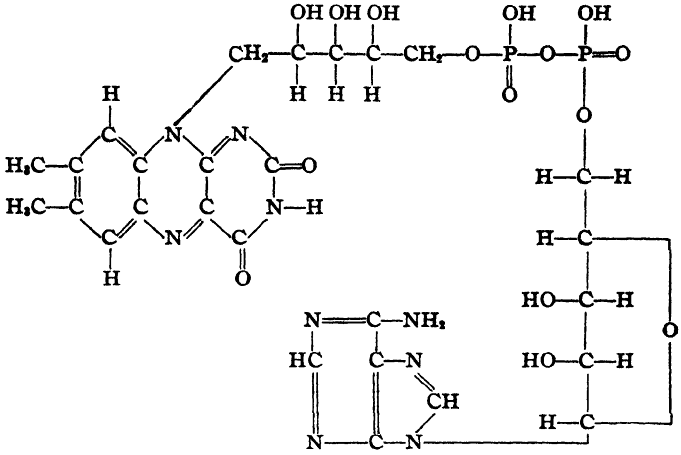
The prosthetic group of the *flavoproteins* ("yellow enzymes") is either flavin-mononucleotide (riboflavinphosphate) or flavin-adeninedinucleotide (coenzyme of the amino acid oxidase). Flavoproteins have been found in the heart muscle, kidney, liver, milk, and yeast. They play an important role in biological oxidation-reduction systems. The action of these flavoproteins appears to be highly specific and consists mainly in the catalysis of the electron transfer between the slow oxidation-reduction systems (pyridine nucleotides) and the cytochrome system of the cells and tissues. (See page 221.)

---

[158] H. v. Euler and F. Schlenk, *Z. physiol. Chem.*, **246**, 64 (1937); F. W. Allen, *Ann. Rev. Biochem.*, **10**, 221 (1941).



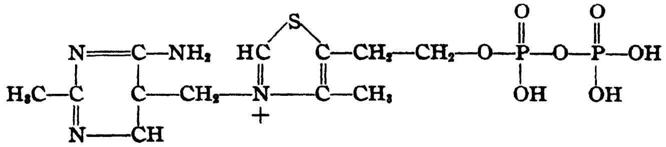
Riboflavinphosphate



Coenzyme of the *d*-amino acid oxidase

This nucleotide, when joined to specific proteins, gives rise to pyridine nucleotide oxidases, xanthine oxidase, and *d*-amino acid oxidase.

Although not a nucleotide, cocarboxylase, which has been isolated in the crystalline state from brewers' yeast, is a pyrimidine derivative. Recent investigations indicate that it plays a similar function in biological oxidation-reduction processes as do some of the nucleotides (*i. e.*, pyridine nucleotides). Cocarboxylase is assigned the following structure:

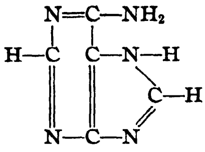


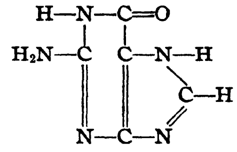
Cocarboxylase (pyrimidine thiazole phosphoric acid)

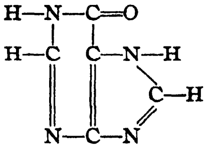
Substances which appear regularly in the nuclei of all plant and animal cells are obviously of great interest to the biochemist. The investigations which led to the observation that chromosomes, tobacco mosaic virus, and other viruses are nucleoproteins[159] emphasizes the biochemical importance of these substances.

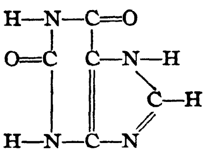
As has been stated before, nucleoproteins are substances formed by the union of nucleic acid and a protein. The following possibilities have been suggested for the type of linkage between the protein and nucleic acid. As the protein component is usually basic in nature, the union between it and the nucleic acid may be of the salt type. The formation of an ester linkage between the thiol group of the protein and the phosphoric acid of the nucleic acid has also been proposed. Generally speaking, experimental evidence indicates that the linkage may be either of the polar or nonpolar type. Another possibility is the existence of coordinate bondage. It has been suggested that the term *nucleoprotein* be reserved for the nonpolar type, while the compounds in which the protein and nucleic acid are united by a polar bond be referred to as *protein nucleates.*

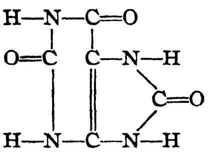
The structures of biochemically important purines and pyrimidines are indicated below:

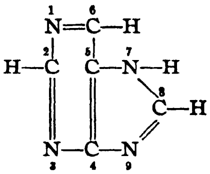

Adenine (6-aminopurine)

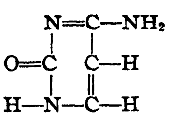

Guanine (2-amino-6-oxypurine)

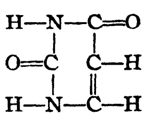

Hypoxanthine (6-oxypurine)

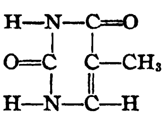

Xanthine (2,6-dioxypurine)

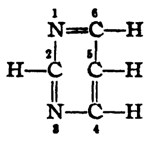

Uric acid (2,6,8-trioxypurine)


Purine


Cytosine (2-oxy-6-aminopyrimidine)


Uracil (2,6-dioxypyrimidine)


Thymine (2,6-dioxy-5-methylpyrimidine)


Pyrimidine

2. Glycoproteins

Glycoproteins (*glucoproteins*) are conjugated proteins containing a prosthetic group which is of carbohydrate nature. The classification and terminology used in connection with this class of substances is far from uniform. Some authors divide this class of substances simply into *mucins* and *mucoids;* others prefer three subclasses, namely,

---

[159] The reader who wishes a more thorough treatment of this subject will find *Nucleic Acids* by Levene and Bass, American Chemical Society Monograph, No. 56, Chemical Catalog Co., New York, 1931, and the review by Johnson in Gilman's *Organic Chemistry,* Vol. 2, p. 950 (1938) instructive reading. For recent contributions, the original literature and the *Annual Review of Biochemistry* should be consulted.

mucins, *mucoids*, and *chondroproteins*. The product of complete hydrolysis, according to the earlier investigators in this field, are *sulfuric acid*, *acetic acid*, a *hexosamine*, and *glycuronic acid* in addition to the protein.

Mucins are slimy substances, insoluble in water or dilute acid. They are secreted by the salivary glands, by mucous membranes, and by the skin of some lower animals. Since they are difficult to obtain in a chemically pure state there is little agreement as to their constitution. Mucoids occur in tendons, *e. g.*, tendomucoid; in bone, *e. g.*, osseomucoid; in eggs, *e. g.*, ovomucoid; and in serum, *e. g.*, serum mucoid. Chondroproteins are derived from cartilage.

Pioneer investigations dealing with the chemical constitution of the prosthetic group revealed the existence of two different conjugated sulfuric acids, depending on the source of the glycoprotein. The conjugated sulfuric acids upon hydrolysis yield sulfuric acid, acetic acid, a hexosamine, and glycuronic acid. Depending on the nature of the carbohydrate group, the conjugated sulfuric acid is either *mucoitinsulfuric acid* or *chondroitinsulfuric acid*. Thus:

Mucoitinsulfuric acid (from gastric mucin, mucin from nasal septa or the cornea) $\xrightarrow{\text{hydrolysis}}$
1. Sulfuric acid
2. Acetic acid
3. 2-Amino galactose (Chondrosamine)
4. Glycuronic acid

Chondroitinsulfuric acid (from cartilage, aorta, sclera, umbilical cord) $\xrightarrow{\text{hydrolysis}}$
1. Sulfuric acid
2. Acetic acid
3. 2-Amino glucose
4. Glycuronic acid

The investigations conducted by Karl Meyer and coworkers have added much to our knowledge of the chemistry of the glycoproteins. Based upon the results of the investigations carried out in his laboratory, Meyer proposes an entirely new classification.

This new classification, together with some pertinent remarks by Meyer, follows:[160]

> "The substances with which we are dealing should not all be classed as proteins. The mucopolysaccharides of our terminology were formerly included as mucins and as mucoids in the group of conjugated proteins. They occur in nature either as free polysaccharides or as protein salts. The second group, the glycoproteins, are polypeptides to which a neutral hexosamine-containing polysaccharide appears to be firmly bound. In bacteria both classes are apparently present, but there remains much to be done in the characterization of definite chemical fractions.
>
> "A logical classification of the hexosamine-containing compounds should be based on the nature of the carbohydrate radical, since the latter is most easily determined. Table I shows the classification which we propose.
>
> "The term 'mucin' is entirely avoided. It should be used only in a physiological sense to denote a viscous fluid of secretory origin. The group of glycoproteins covers mucoids in general, such as serum or ovomucoid, but will also include the carbohydrate-containing globulins such as serum and ovoglobulin, thyroglobulin, etc. It is not at all certain whether the latter are chemical entities or molecular compounds of protein with mucoids. . . .
>
> "Glycoproteins contain hexosamine bound to protein or polypeptide in addition to other sugars.

---

[160] The classification (Table I) as well as the quotations are taken from Meyer's review on "The Chemistry and Biology of Mucopolysaccharides and Glycoproteins," *Cold Spring Harbor Symposia on Quantitative Biology*, 6, 91, 99 (1938). The reader is urged to consult this review.

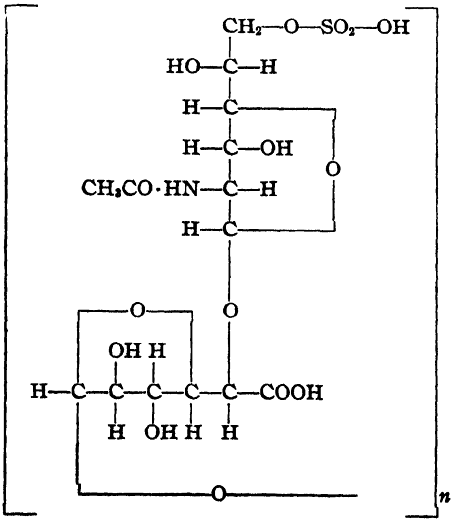
"Table I* (Meyer)

- A. Mucopolysaccharides
  - I. Containing uronic acid
    - (*a*) Sulfate-free
      - 1. Vitreous humor, umbilical cord, synovial fluid, group A hemolytic streptococcus
      - 2. Type I pneumococcus
    - (*b*) Sulfate-containing
      - 1. Chondroitinsulfuric acid
      - 2. Mucoitinsulfuric acid (from gastric 'mucin' and cornea)
      - 3. Heparin
  - II. Neutral mucopolysaccharides of known composition
    - (*a*) Chitin
    - (*b*) Gastric polysaccharide
    - (*c*) Bacterial polysaccharides (Shiga-Kruse (?), Type IV pneumococcus (?))
- B. Glycoproteins, containing neutral mucopolysaccharides of unknown composition
  - (*a*) Ovomucoid-α (formerly called ovomucoid)
  - (*b*) Ovomucoid-β (formerly called ovomucin)
  - (*c*) Serum mucoid, serum glycoid
  - (*d*) Globulins (egg white, serum, thyroglobulin)
  - (*e*) Pregnancy urine hormone"

---

* The author of this table uses the expression uronic acid in place of glycuronic acid.

According to investigations carried out in Meyer's laboratory, the protein complexes of chondroitinsulfuric acid are salts. The formation of the salt takes place by the interaction of a basic group of the protein with the acid group of the chondroitinsulfuric acid. The tentative formula for chondroitinsulfuric acid is indicated below:



3. Phosphoproteins

Phosphoproteins are usually considered to be "components of the protein molecule with phosphorus-containing substances other than nucleic acid or lecithin." They may be differentiated from nucleoproteins by the fact that they do not yield purines and pyrimidines upon hydrolysis. The phosphorus appears to be a constituent of the phosphoric acid radical, the latter being united with the protein molecule by way of an ester linkage.

Phosphoproteins are important constituents of the food of the young animal and growing bird. The best-known members of this class of proteins are casein and ovovitellin. These substances are not homogeneous but may be separated into various components by means of appropriate solvents.

Recent investigations indicate that casein added to the diet reduces the amount of fat deposition in the liver. Other proteins possess lipotropic activity as well, but (with the exception of whale-muscle protein) not to the same extent as casein. The lipotropic action of casein is due probably to the constituent amino acids and should not be assigned to the prosthetic group.

4. Chromoproteins

Chromoproteins are usually defined as compounds of a protein and *heme* (*hematin*) or a substance closely related to it. The most important members of this class of compounds are the *hemoglobins*.

Hemoglobins are composed of a protein, globin, and the prosthetic groups, *heme* (an iron containing *porphyrin* complex). The prosthetic group derived from these compounds has been studied, particularly by Hans Fischer and *hemin*, the chloride of heme, has been synthesized by him.

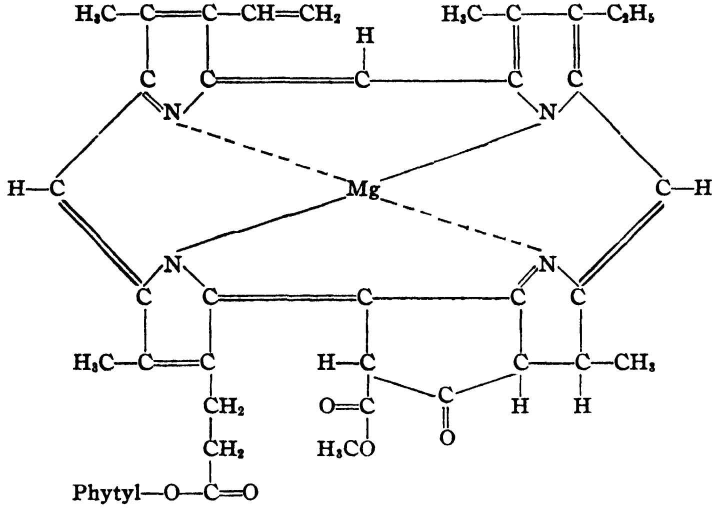
The free acidic groups in heme form the means by which it may unite with the protein molecule to give rise to the important respiratory pigments.

The protein moiety of the mammalian hemoglobin has been studied more thoroughly than the protein derived from the chromoproteins found in invertebrates. Investigations carried out by Block, Vickery, and others indicate that the molecular ratios of tryptophane, tyrosine, arginine, histidine, and lysine found in the globins derived from different mammalian hemoglobins are practically the same. No such constancy in the values of the molecular ratios is observed in the case of sulfur, cystine, and methionine.

The protein moiety found in the respiratory pigments of invertebrates has not been sufficiently studied to warrant any general conclusions. The respiratory pigment *helicorubin* found in pulmonate mollusks appears to be composed of a globin and heme. Similarly, *chlorocruorin* (found in certain marine worms) and *actiniohematin* (found in actinia) are also respiratory pigments which resemble hemoglobin with respect to chemical constitution and physiological properties.

Another important porphyrin complex is chlorophyll. Certain experimental observations indicate chlorophyll to exist in combination with the protein found in the chloroplasts. Assuming this view to be correct, the chlorophyll-protein complex must be regarded as a chromoprotein.

The pioneer investigations conducted by Willstätter and others revealed two modifications of this important plant pigment, chlorophyll *a* and chlorophyll *b*. Both, heme and chlorophyll, on reduction and oxidation, yield pyrrole compounds; furthermore, the treatment of chlorophyll or heme with alkali leads to the formation of porphyrins. Thus, the study of the chemical properties as well as the absorption spectra of heme, chlorophyll, and their respective derivatives indicates a close relationship between the prosthetic group of hemoglobin and the porphyrin complex found in the chloroplasts. Hans Fischer proposed the following structure for chlorophyll *a:*



While iron is the metal present in heme, magnesium is found in the case of chlorophyll.

The addition of *denatured globin* to reduced *heme* gives rise to a compound known as hemochromogen.[161] The addition of other nitrogenous compounds, such as proteins other than globin, (*e. g.*, albumin), pyridine, nicotine, cyanide, ammonia, etc., leads to the formation of compounds which give absorption spectra related to hemochromogen. The compounds are known as *hemochromogens.* Since the absorption spectra of the various members of this class of compounds are not identical, they may be differentiated from each other by means of spectroscopic examination. A number of naturally occurring compounds belong to the class of protein-hemochromogens. *Cytochrome oxidase* (formerly known as *indophenol oxidase*) is a hemochromogen. It is present in all animal and plant cells and is closely connected with the activity of the *cytochromes.* Likewise, the substances known as cytochromes are conjugated proteins containing a heme complex. They are present in all living cells of aerobic organisms and apparently play a paramount role in biological oxidative processes. Summer and Dounce have succeeded in the preparation of crystalline *catalase.* This enzyme, which is found in plant and animal tissues, is involved in the decomposition of hydrogen peroxide into water and molecular oxygen. It also is a protein-hemochromogen. The spectroscopic examination of *peroxidase* (extracted from horse-radish) reveals a similar structure.

---

[161] Native globin and heme react to form hemoglobin.

Hemocyanins are copper-containing respiratory pigments found in the blood of some invertebrates, notably in the case of mollusks (*e. g.*, cephalopods, gastropods) and crustaceans. The respiratory pigments have a blue color when in the oxidized state. Formerly they were regarded as conjugated proteins, possessing a prosthetic group composed of a copper porphyrin complex; experimental observations, however, fail to indicate the presence of a copper-porphyrin group.

The prosthetic group of *lecithoproteins* is *lecithin*, *cephalin*, or some similar substance, *i. e.*, phospholipids (see page 100). This group of conjugated proteins has not been subjected to exhaustive investigations, and our knowledge of the chemical and physiological properties is but fragmentary.

The phospholipid-protein complexes are widely distributed in plant and animal matter. Thus, lipoproteins (a more inclusive term) occur in cell membranes, in milk, in egg yolk, in blood, in cell nuclei, and in chloroplasts. In addition, lipoproteins have been found present in bacterial antigens and viruses.

As has been previously stated, lipoproteins may act as haptens in the determination of serological specificity. The occurrence of phospholipid-proteins in the blood suggests the mechanism for the transportation of phospholipids. The conversion of prothrombin to thrombin, according to recent investigations, appears to be catalyzed by a lipoprotein present in lung tissue. Cephalin (between *p*H 2 and 7) and lecithin (between *p*H 7 and 8) form insoluble compounds with thymus histone. Since cephalin is essential to the process of blood clotting, the ability of histone to inhibit blood coagulation may be due to the formation of the insoluble lipoprotein.

Synthetic lipoproteins have been prepared by the addition of alcoholic solutions of lecithin to solutions of serum albumin.

Experimental evidence available at the present time does not permit the indication of the mode of linkage between the prosthetic group and the protein molecule. It has been suggested that the union takes place by way of a nonpolar valence bond.

XVI. PROTEINS AND AMINO ACIDS IN NUTRITION

While the higher animals are unable to synthesize amino acids (with the possible exception of glycine) at a rate and in quantity adequate for maintenance and growth, plants are capable of producing these substances from carbon dioxide, water, and nitrates. The amino acids are then converted into proteins, and it is in this form that they become available as a source of amino acids. From these the animals produce the proteins present in all the living cells.

It is obvious, therefore, that proteins are an indispensable requirement in the diet of man. Since the ingested proteins are not utilized directly, but split into their constituent amino acids by the action of proteolytic enzymes, the nutritive value depends on the kinds and amounts of the amino acid radicals they contain.

1. Feeding Experiments

From the pioneer work of Osborne and Mendel it became evident that proteins differ widely with respect to their nutritional values. These investigators soon recognized that some proteins sufficed to support maintenance and growth, whereas others were inadequate even for maintenance.

Feeding experiments in which young rats received food mixtures containing 18% casein, gliadin, or zein, respectively, as the sole protein indicated that casein sufficed to support growth; gliadin sufficed for maintenance and very slight growth; zein was inadequate even for maintenance. Further experiments showed that upon the addition of lysine to the gliadin diet, the latter sufficed to support growth. Similarly, the zein diet became adequate for maintenance when tryptophane was added, whereas the addition of tryptophane and lysine sufficed to support growth.[162]

While casein[163] fed as 18% of the diet supported growth at a normal rate, a much slower rate of growth was observed when casein was present to the extent of 9% of the food mixture. This time the limiting factor proved to be cystine.

The early experimental method, therefore, was to add a single, purified protein (in known quantity) to the food mixture and observe the rate of growth of the experimental animal. "Deficient" proteins were then supplemented with the missing amino acid.

While feeding experiments with rations containing a single protein give information as to the relative adequacy of the particular protein as a source of "essential" amino acids, the diet under normal conditions furnishes more than one protein. By feeding mixtures of pure proteins of known composition, Mendel and Osborne have shown how proteins supplement each other in nutrition.

Zein is inadequate as the sole source of amino acids; lactalbumin, while adequate to support rapid growth when fed to the extent of 8% of the food ration, supports only slow growth when constituting 4.5% of the food mixture. A diet containing 13.5% of zein and 4.5% of lactalbumin, however, will support normal growth. Thus, lactalbumin furnishes the amino acids which zein lacks; zein, which does not even suffice for maintenance, supplies the constituents necessary to maintain rapid growth with a low lactalbumin intake.

Thus, whereas cereal proteins are inadequate sources of the "essential" amino acids, a feeding mixture containing cereal grain supplemented with the proper ration of milk may be entirely adequate. Since milk is a rich source of tryptophane and lysine, it supplies the essential factors which are lacking in cereals.

The observations of Osborne and Mendel[164] showed that (*a*) growth can be maintained with certain proteins but not with others; (*b*) the nutritive value of a single protein is determined by the amount of its least abundant constituent amino acid; (*c*) proteins supplement each other in nutrition.

These experiments further indicated that casein (from milk), lactalbumin (from milk), ovalbumin (from hen's egg), ovovitellin (from hen's egg), cannabin (from hempseed), edestin (from hempseed), excelsin (from Brazil nut), globulin (from cottonseed or squashseed), glutenin (from maize or wheat), and glycinin (from soybean) sufficed for growth when fed singly in adequate concentrate; while conglutin (from blue or yellow lupin), gelatin (from horn), gliadin (from wheat or rye), hordein (from barley), legumin (from pea or vetch), legumelin (from soybean), phaseolin (from white kidney bean), vignin (from vetch), and zein (from maize) were inadequate to maintain growth when they were fed singly and constituted the sole source of amino acids.

---

[162] L. B. Mendel, *J. Am. Med. Assoc.*, **64**, 1539 (1915)

[163] Casein is deficient in glycine. Since this amino acid can be synthesized by the animal organism, this deficiency is not serious from the nutritional point of view.

[164] T. S. Osborne and L. B. Mendel, *J. Biol. Chem.*, **12**, 81 (1912); **15**, 311 (1913); **16**, 423 (1913); **17**, 325 (1914).

Protein digests composed of amino acids have also been used for nutritional studies. After the removal of particular amino acids from the digest, the latter may be used for nutritional studies.

Using this procedure, Berg and Rose[165] found that tryptophane could be replaced by synthetic 3-indolepyruvic acid. As early as 1926, Cox and Rose[166] demonstrated that *dl*-β-4-imidazolelactic acid induced growth, when added to a histidine-deficient diet.

The experiments of Rose and coworkers[167] in connection with the nutritional importance of histidine and arginine, conclusively showed that (*a*) histidine is an "essential" amino acid; (*b*) arginine and histidine are not interchangeable; (*c*) arginine can be synthesized in the animal body, but at too slow a rate to support normal growth.

The use of a single protein or a protein digest in nutritional experiments was followed by the development of a new technique. Rose adopted the procedure of feeding a mixture of purified amino acids in place of the single protein or protein digest. This eliminated the uncertainty as to the composition of the amino acid source; that is, the possibility of the presence of "essential" amino acids of unknown constitution or of small amounts of known structure was eliminated.

Rose[168] found that a mixture of 19 highly purified amino acids commonly occurring in protein (*e. g.*, casein) does not permit even maintenance of body weight. These observations seemed to indicate the presence of an essential factor or factors in the natural sources. The extensive researches carried out by Rose and coworkers[169] led to the discovery of threonine (α-amino-β-hydroxy-*n*-butyric acid). The synthetic mixture of 19 amino acids and threonine did support rapid growth.

By using a mixture of purified amino acids as the sole source of nitrogen, Womack, Kemmerer, and Rose[170] demonstrated that cystine is not an "essential" amino acid, while methionine must be regarded as indispensable.

According to Rose, the amino acids may be classified as essential (indispensable) and nonessential (dispensable). Rose defines an essential amino acid as one which cannot be synthesized by the animal organism at a rate commensurate with the demands for normal growth.

---

[165] C. P. Berg and W. C. Rose, *J. Biol. Chem.*, **82**, 479 (1929).

[166] W. M. Cox and W. C. Rose, *J. Biol. Chem.*, **68**, 781 (1926).

[167] W. C. Rose and G. J. Cox, *J. Biol. Chem.*, **61**, 747 (1924); **68**, 217 (1926). W. E. Bunney and W. C. Rose, *ibid.*, **76**, 521 (1928). C. W. Scull and W. C. Rose, *ibid.*, **89**, 109 (1930).

[168] W. C. Rose, *J. Biol. Chem.*, **94**, 155 (1931).

[169] W. Windus, F. L. Catherwood, and W. C. Rose, *J. Biol. Chem.*, **94**, 173 (1931). C. T. Caldwell and W. C. Rose, *ibid.*, **107**, 45, 57 (1934). M. Womack and W. C. Rose, *ibid.*, **112**, 275 (1935). R. H. McCoy, C. E. Meyer, and W. C. Rose, *ibid.*, **112**, 283 (1935). W. C. Rose, *Physiol. Revs.*, **18**, 109 (1938).

[170] M. Womack, K. S. Kemmerer, and W. C. Rose, *J. Biol. Chem.*, **121**, 403 (1937).

(a) *Essential Amino Acids*

Essential amino acids include lysine, tryptophane, histidine, phenylalanine, leucine, isoleucine, threonine, methionine, valine, and arginine. See page 257.

Rose also indicates the minimum amount of each essential amino acid necessary to support normal growth when the nonessentials are included in the food ration. These amounts, given in per cent of food consumed, are as follows: lysine, 1.0%; leucine, 0.9%; phenylalanine, 0.7%; valine 0.7%; threonine, 0.6%; methionine, 0.6%; isoleucine, 0.5%; histidine, 0.4%; tryptophane, 0.2%; and arginine, 0.2%.

A series of investigations dealing with the relative nutritional value of the isomeric forms of the amino acids shows that histidine, methionine, phenylalanine, and tryptophane can be used with equal efficaciousness in the *d*-, *l*-, or *dl*-forms.

(b) *Nonessential Amino Acids*

Nonessential amino acids include glycine, alanine, serine, norleucine, aspartic acid, glutamic acid, hydroxyglutamic acid, proline, hydroxyproline, citrulline, tyrosine, and cystine.

TABLE 22

PROXIMATE PROTEIN CONTENT OF SOME FOODS[a]

Food	Protein, %	Food	Protein, %
	GROUP I (*a*) Meats		
Bacon, raw	10.5	Goose	16.3
Bacon, cooked	21.3	Ham, fresh, lean	25.0
Beef, miscellaneous cuts, lean	22.4	Ham, smoked, medium fat	16.3
Beef, fatty	16.2	Lamb chops, edible portion	18.7
Beef, lean roast	23.0	Lamb, roast	19.7
Beef, dried	30.0	Pork chops, lean	20.3
Bologna sausage	17.6	Pork chops, medium fat	16.6
Chicken, edible portion	21.5	Pork, sausage	16.7
Frankfurters	19.6	Veal chops, medium fat	19.9
	(*b*) Fish		
Codfish, fresh	16.7	Salmon, fresh	22.0
Codfish, salt, cooked	42.3	Salmon, canned	21.8
Halibut	18.6	Sardines	23.0
Oysters	6.2	Whitefish	22.9
	(*c*) Dairy Products		
Butter	1.0	Ice cream	2.1
Buttermilk	3.0	Milk, whole	3.3
Cheese	27.7	Milk, skim	3.4
Cheese, cottage	20.9	Milk, evaporated	9.6
Cream, 20%	2.8	Milk, condensed	8.8
	(*d*) Eggs		
Eggs, whole	13.4	Eggs, yolk	15.3
Eggs, white	12.3		

TABLE 22 (*Continued*)

PROXIMATE PROTEIN CONTENT OF SOME FOODS

GROUP II

(*a*) Cereals (Breakfast Foods) and Breads

Food	Protein, %	Food	Protein, %
Bread, graham	8.9	Crackers, soda	6.6
Bread, rye	9.0	Oatmeal, uncooked	16.7
Bread, white, milk	9.6	Oatmeal, cooked	2.8
Bread, whole wheat, milk	9.7	Puffed rice	8.0
Cornbread	6.2	Puffed wheat	13.0
Cornflakes	5.5	Shredded wheat	12.1
Crackers, graham	10.0	Zwieback	9.8

(*b*) Fruits, Berries, and Nuts

Food	Protein, %	Food	Protein, %
Almonds, edible portion	21.0	Oranges, edible portion	0.9
Apples, fresh	0.4	Peaches, canned	0.7
Apricots, edible portion, fresh	1.0	Peaches, edible portion, fresh	0.5
Apricots, dried	4.7	Peanuts	25.8
Bananas, edible portion	1.2	Pears	0.7
Blackberries	1.2	Pineapples, edible portion, fresh	0.4
Blueberries	0.6	Plums, edible portion, fresh	0.7
Cherries, edible portion	1.3	Prunes, edible portion, fresh	2.1
Chestnuts	6.3	Prunes, dried, cooked	0.8
Cranberries, including refuse	0.4	Raisins	2.6
Gooseberries	0.8	Raspberries, black, fresh	1.5
Grapes	1.4	Strawberries, canned	0.7
Grapefruit, edible portion	0.5	Strawberries, fresh	0.8
Loganberries	1.0	Walnuts, black, edible portion	27.6
Olives, ripe	1.4	Walnuts, English, edible portion	18.4

(*c*) Vegetables and Legumens

Food	Protein, %	Food	Protein, %
Asparagus	1.8	Lettuce	1.2
Beans, navy, cooked	7.5	Peas, fresh	7.0
Beans, navy, dried	22.5	Peas, dried, cooked	6.6
Beans, soya	37.0	Potato, boiled	2.5
Beans, Lima, edible portion, fresh	7.1	Potato, baked	3.1
Beans, Lima, dried	18.1	Potato, mashed	2.5
Beans, string, raw	2.3	Potato, fried	2.3
Beets	1.6	Potato, sweet, baked	2.1
Cabbage	1.6	Sauerkraut	1.7
Carrots	1.1	Spinach, cooked	2.1
Cauliflower, edible portion	1.8	Squash, edible portion	1.4
Celery	1.1	Tomato, raw, including refuse	0.9
Corn, canned	3.1	Tomato, canned	1.2

---

[a] The data listed in Table 22 have been selected from values reported in N. A. Lange and G. M. Forker, *Handbook of Chemistry*. 4th ed., Handbook Pubs., Sandusky, 1941, pp. 562, 564, 566, and 568.

2. Application of Heavy Isotopes

In more recent investigations dealing with the intermediate metabolism of amino acids, use has been made of radioactive sulfur, deuterium, and isotopic nitrogen.

Travers and Schmidt,[171] employing radioactive sulfur, have shown methionine to be the precursor of cystine.

Schoenheimer and associates,[172] using deuterium-labeled phenylalanine, observed the conversion of phenylalanine into tyrosine. Moss and Schoenheimer,[173] adding *d*-leucine labeled with deuterium and isotopic nitrogen to the food ration, have demonstrated its partial conversion to the natural form (*l*-leucine).

It is obvious, from what has been said above, that an adequate diet must include proteins which furnish all the essential amino acids in sufficient amounts. It has been suggested that an allowance for an adult of approximately 1 g. of mixed proteins per kg. of body weight is adequate, provided, of course, that the other components of the diet meet the energy requirements of the organisms. Since animal proteins are rich in "essential" amino acids, it has been recommended that about 70% of the protein intake should consist of meat, milk, eggs, or fish. While cereals do contain proteins, the latter may be deficient in essential amino acids. Most vegetables contain relatively small amounts of proteins.

XVII. RECENT DEVELOPMENTS

While the limited space available for this chapter does not permit the outline of all the recent contributions to the various phases of the chemistry of the amino acids and proteins, it is appropriate to cite a few reviews and publications dealing with some of the subject matter not treated or only touched upon lightly. In this connection, the reader should refer to the recently published *Addendum to the Chemistry of the Amino Acids and Proteins*[174] and to the *Annual Review of Biochemistry*.[175]

The past few years have witnessed the publication of a large number of papers dealing with the isolation of amino acids and proteins, the synthesis of amino acids and methods of analysis. The use of the solubility method of Bergmann and Stein,[176] as well as the isotope dilution method of Rittenberg and Foster[176a] have been mentioned elsewhere in this chapter (pages 156, 157). Reference to these contributions is made again because of their importance as new procedures for the determination of amino acids. The method for the synthesis of polypeptide chains, as developed by Bergmann and Zervas,[177] as well as the method for the analysis of peptide chains[178] have also been dealt with.

The monumental work of Bergmann and coworkers on the use of synthetic polypeptides for the characterization of proteolytic enzymes is discussed on page 212 in this volume.

---

[171] H. Travers and C. L. A. Schmidt, *J. Biol. Chem.*, **130**, 67 (1939).

[172] S. Ratner, R. Schoenheimer, and D. Rittenberg, *J. Biol. Chem*, **134**, 653 (1940).

[173] A. R. Moss and R. Schoenheimer, *J. Biol. Chem.*, **135**, 415 (1940).

[174] C. L. A. Schmidt, *Addendum to the Chemistry of the Amino Acids and Proteins.* Thomas, Springfield, 1943.

[175] J. M. Luck, *Annual Review of Biochemistry.* Vols. I–XI, Annual Reviews, Inc., Stanford Univ., Calif., 1932–1942.

[176] M. Bergmann and W. H. Stein, *J. Biol. Chem.*, **129**, 609 (1939).

[176a] D. Rittenberg and G. L. Foster, *J. Biol. Chem.*, **133**, 737 (1940).

[177] M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

[178] M. Bergmann and L. Zervas, *J. Biol. Chem.*, **113**, 341 (1936).

Doubt has been cast on the validity of some of the hypotheses concerning the structure of proteins. This is particularly true of Sørensen's concept that proteins may be regarded as reversible dissociable component systems,[179] of Bergmann's and Niemann's periodicity hypothesis,[180] and of the cyclol theory as proposed by Wrinch.[181] For a critical review of these hypotheses, the reader should refer to the excellent chapter by Bull.[182] With reference to his basic amino acid "Anlage" hypothesis,[183] Block suggests that the basic amino acids may play an especially important role in the genetic development of the eukeratins.[184] The last-named hypothesis, thus, has but limited application.

The use of isotopes for the tracing of the intermediate metabolism of amino acids and proteins must be regarded as a major contribution. The reviews by Schoenheimer and Rittenberg[185] and Schoenheimer and Rattner[186] are particularly noteworthy.

Stiller and coworkers[187] have recently reported the synthesis of pantothenic acid. This acid, which has been classified as a vitamin, may be regarded as a peptide consisting of α,γ-dihydroxy-β,β'-dimethylbutyric acid and β-alanine. Hydroxypantothenic acid[188] also possesses biological activity.

Recent investigations indicate that some of the vitamins of the B complex constitute the prosthetic group of certain proteins. These conjugated proteins appear to play an active role in some of the biological oxidation processes.[189]

Heidelberger[190] has summarized recent advances dealing with the chemical nature of antigens and antibodies.

---

[179] S. P. L. Sørensen, *Compt. rend. trav. lab. Carlsberg,* **18** (No. 5), 1 (1930).

[180] M. Bergmann, *Harvey Lectures.* Vol. 31, Williams & Wilkins, Baltimore, 1936, p. 37. M. Bergmann and C. Niemann, *J. Biol. Chem.*, **118,** 301 (1937); **122,** 577 (1938).

[181] D. N. Wrinch, *Nature,* **137,** 411 (1936); **138,** 241, 651, 758 (1936); *Proc. Roy. Soc. London,* **A160,** 59 (1937); *Trans. Faraday Soc.,* **33,** 1368 (1937).

[182] H. B. Bull, "Protein Structure," in F. F. Nord and C. H. Werkman, *Advances in Enzymology,* Vol. 1. Interscience, New York, 1941.

[183] R. J. Block, D. C. Darrow, and M. K. Carey, *J. Biol. Chem.,* **104,** 347 (1934). R. J. Block, *ibid.,* **105,** 445 (1934).

[184] R. J. Block in C. L. A. Schmidt's *Addendum to the Chemistry of the Amino Acids and Proteins.* Thomas, Springfield, 1943, p. 1101.

[185] R. Schoenheimer and D. Rittenberg, *Physiol. Revs.,* **20,** 218 (1940).

[186] R. Schoenheimer and S. Rattner, *Annual Review of Biochemistry.* Vol. X, Annual Reviews, Inc., Stanford Univ., Calif., 1941, p. 197.

[187] E. T. Stiller, S. A. Harris, J. Finkelstein, J. C. Keresztesy, and K. Folkers, *J. Am. Chem. Soc.,* **62,** 1785 (1940).

[188] H. K. Mitchell, E. E. Snell, and R. J. Williams, *J. Am. Chem. Soc.,* **62,** 1791 (1940).

[189] E. S. G. Barron, *Annual Review of Biochemistry.* Vol. X, Annual Reviews, Inc., Stanford Univ., Calif., 1941, p. 1.

[190] M. Heidelberger in C. L. A. Schmidt's *Addendum to the Chemistry of the Amino Acids and Proteins.* Thomas, Springfield, 1943, p. 1258.

Selected Bibliography

Block, R. J., and Bolling, D., *The Determination of the Amino Acids.* Burgess, Minneapolis, 1940.

Cannan, R. K., Kibrick, A., Kirkwood, J. G., Longsworth, L. G., Palmer, A. H., and Steinhardt, J., "Amphoteric Properties of Proteins," *Ann. N. Y. Acad. Sci.*, **41**, 241 (1941).

Cohn, E. J., and Edsall, J. T., *Proteins, Amino Acids and Peptides.* Reinhold, New York, 1942.

Cohn, E. J., Fankuchen, I., Oncley, J. L., Vickery, H. B., and Warren, B. E., "Crystalline Protein Molecules," *Ann. N. Y. Acad. Sci.*, **41**, 77 (1941).

Gilman, H., *Organic Chemistry.* Wiley, New York, 1943.

Meyer, K. H., *Natural and Synthetic High Polymers.* Interscience, New York, 1942.

Mitchell, H. H., and Hamilton, T. S., *The Biochemistry of the Amino Acids.* Chemical Catalog Co., New York, 1929.

Schmidt, C. L. A., *The Chemistry of the Amino Acids and Proteins.* Thomas, Springfield, 1938.

Schmidt, C. L. A., *Addendum to the Chemistry of the Amino Acids and Proteins.* Thomas, Springfield, 1943.

Chapter VI

ENZYMES

KURT G. STERN

New York, N. Y.

I. INTRODUCTION

The most widely accepted definition for an enzyme appears to be that given by Haldane.[1] He defines an enzyme as a *soluble, colloidal, organic catalyst which is produced by a living organism.* This definition excludes such low-molecular weight catalysts as glutathione, ascorbic acid (vitamin C), or pyocyanine, as well as the catalytically active surfaces which undoubtedly exist in certain regions such as interfaces of the cell but which have not yet been sufficiently explored and which, by their very nature, cannot be separated from the cellular structure.

The great majority of enzymes are present in the interior of cells from which they may be released either by voluntary, physiological secretion processes, by autolysis, *i. e.*, postmortal decomposition, or by deliberate crushing and destruction of the cell structure, such as grinding with or without abrasives, intense sonic or ultrasonic vibrations and other processes. Some enzymes may be isolated from natural secretions such as gastric juice (pepsin), pancreatic secretion (trypsin complex), and milk (peroxidase, xanthine oxidase).

Among the characteristic properties of enzymes are their great catalytic activity as compared with their concentration, their pronounced specificity, the dependence of their activity upon the hydrogen-ion concentration, and their thermolability. It is likely that not only the two last-named but all of the characteristics of enzymes are related to their *protein* nature. While it may safely be stated that all enzymes thus far described are either "simple" or conjugated proteins, not all proteins endowed with catalytic activity are necessarily enzymes. A case in point is hemoglobin and other respiratory transport proteins which possess, in addition to their main function of reversible gas-binding, weak and presumably incidental catalytic activity toward hydrogen peroxide.

The protracted controversy as to whether or not enzymes are genuine catalysts, may be considered settled in favor of the thesis that they are true catalysts.

---

[1] J. B. S. Haldane, *Enzymes*, Longmans, Green, London, 1930; J. B. S. Haldane and K. G. Stern, *Allgemeine Chemie der Enzyme*, Steinkopff, Dresden, 1932.

II. ENZYMES AS CATALYSTS

To state that the enzymes are indispensable for the functioning of the cell and for life in general today amounts to a commonplace. Their nature as true catalysts implies that they should be able to catalyze a given process not only in the sense of the forward reaction but also in the direction of the back reaction. In other words, if the forward reaction represents a breakdown of a substrate into smaller units, the enzyme should also be able to promote the resynthesis of these elementary units into the original, large molecule. While the synthetic power of enzymes has thus far been demonstrated experimentally only in a relatively small number of technically favorable instances, *e. g.*, formation of fatty esters and carbohydrates, there is little room for doubt that within the living cell one and the same enzyme participates in anabolic as well as catabolic processes. The extent and rate of these reactions taking place continuously during cell life are probably governed less by the position of equilibria than by the state of activation of the enzyme, by the rate of enzyme production from inactive precursors, by the relative concentrations of the various reactants, and, last though not least, by spatial separation of enzyme and substrate within the architecture of the cell. At best, the situation in the cell might be described as a steady state; the attainment of a true equilibrium state within the cell is probably synonymous with death. The enzymatic apparatus of the cell is elastic in that the formation of active enzymes from their precursors keeps pace with the demands within wide limits. Furthermore, there is evidence to show that cells can produce enzymes of the desired specificity if faced with a substrate of an unfamiliar type. It is logical to assume that the overwhelming number of different catalytic activities encountered in cells is the result of the combination, variation, or permutation of a limited number of building blocks or of minor modifications of a few basic types of structures and catalytic faculties. This hypothesis receives strong support from the observation of the gradual appearance and diversification of enzymatic functions in the course of the development and maturation of higher organisms. Like the other organic constituents of protoplasm, the enzyme is a perishable factor, its life span being determined by the concentration and activity of other enzymes to which some of its component parts, such as its protein moiety or phosphate bonds, may fall prey and also by the extent to which it becomes involved in the reactions which it is able to catalyze. It is a well-known fact that a given amount of enzyme material, if added to a solution of its substrate even under optimum conditions for its action, becomes "exhausted" after a certain and usually large quantity of the substrate has been transformed into the reaction products. This observation has given rise to the suspicion that enzymes may not be true catalysts but that they are unstable substances the spontaneous breakdown of which induces the decomposition of a large number of substrate molecules. This explanation of enzymatic processes as induced reactions is reminiscent of Liebig's theory of enzyme action. Today we know that the inactivation of enzymes occurring during their functioning is not intrinsically coupled with the mechanism of their action but is due to incidental and irreversible side reactions which lead to the gradual destruction of the enzyme molecule, *e. g.*, by reaction products, without maintaining any stoichiometric relationship to the main reaction, namely, the enzymatic catalysis. The equally old observation that enzyme preparations become, in general, increasingly labile upon progressive purification—so much so that some of the most highly purified preparations, when kept in solution, may appear catalytically inactive upon assay—and which led Willstätter[2] to the postulate in 1926 that enzymes do not belong to any of the known classes of chemical compounds, has since found a rational explanation. In their natural environment, the enzymatic proteins are usually protected to a certain degree against thermal inactivation, irreversible inactivation, or inhibition by heavy metals. This protection is provided by inert proteins and substances forming complexes with heavy metals (polypeptides, amino acids, organic acids in general, carbohydrates, etc.). It is readily seen that the removal of such adventitious substances upon purification of the enzyme will render the latter more and more open to attack by a variety of physical and chemical factors.

Chemical and technological food research projects are rarely concerned with problems relating to the chemical nature or the action-mechanism of enzymes. The type of information desired is either qualitative in nature, relating to the presence or absence of certain enzymes, or quantitative data on the concentration of enzymes in foodstuffs or their sources. Furthermore, the effect of certain factors and treatments, *e. g.*, refrigeration, cooking, or dehydration, on the activity of enzymes is frequently the object of inquiry. The minute absolute concentration in which enzymes usually occur in biological materials precludes the direct, analytical approach, *i. e.*, tests for the enzymes as chemical individuals. Just as vitamins and hormones are frequently identified by bioassay techniques—involving the determination of their specific biological activity in suitable test objects—the enzyme content of food materials is as a rule estimated by studying the *rate* at which a given specific substrate is attacked by the material under investigation or by extracts prepared from it. The result of such estimations is then expressed in terms of *enzyme units* which have previously been defined in a more or less arbitrary manner and frequently without knowing as to which actual absolute quantity of an enzyme corresponds to the reaction rate observed under the standardized conditions of the test. This defect, however, is not very serious since only relative rather than absolute enzyme concentrations are ordinarily of interest. This is particularly true in those many instances where the enzyme under study has thus far not been isolated in pure form and its chemical structure is obscure. In other cases the nutrition chemist employs enzymes merely as specific tools to prepare a biological material for analytical investigations. Thus several vitamins (biotin and pyridoxine, etc.) occur in their natural environment partly in bound form, probably linked to proteins; and it becomes necessary to liberate the vitamin by the use of suitable enzyme preparations prior to their colorimetric assay. For such purposes it is often convenient and perfectly legitimate to use commercial enzyme preparations of the desired specificity, even though their degree of purity and chemical composition may be unknown.

---

[2] R. Willstätter, *Ber.*, 59, 1 (1926).

Inasmuch as the vast majority of enzyme determinations are based on studies of reaction rates, a knowledge of the *kinetics* of enzymatic catalyses and of the factors influencing the velocity of these reactions is a prerequisite for the judicious choice of methods and for the critical evaluation of the results obtained. While the rate of reactions, catalyzed by enzymes, thus assumes a paramount interest from a practical point of view, we must not forget that enzymes, just as any other catalysts, can do more than just accelerate thermodynamically possible reactions, *viz.*, processes occurring with a loss in free energy. They can also influence the *direction* of such reactions and thus determine the nature of the product molecules. This explains why one and the same substance, when subjected to the action of different enzymes, may yield materials of very different structure. Pyruvic acid, for example, is transformed anaerobically into acetaldehyde and carbon dioxide under the influence of carboxylase and into acetic acid and carbon dioxide by oxidative decarboxylation through pyruvic acid dehydrogenase. This effect of enzymes, which at first glance appears to conflict with our earlier views that enzymes (and catalysts in general) merely speed up reactions already proceeding spontaneously at a small rate, is readily explained by assuming that the uncatalyzed process is capable of proceeding in different directions with rates which are unequally affected by different enzymes. Let us assume that a substance, A, in the absence of a catalyst, yields upon decomposition 99% of the product B and 1% of C. If the catalyst accelerates the latter reaction a million-fold, A will now yield 99.99% of compound C and only 0.01% of B. Another enzyme may promote another side reaction and thus change the proportion and nature of reaction products. One of the most versatile substrates in this regard is pyruvic acid which is transformed into a large number of different products depending on the enzyme employed.

1. Kinetics of Enzyme Reactions

In discussing the factors involved in measurements of reaction velocities, as influenced by enzymes, we have to distinguish between experiments designed to obtain information of the degree of activity of a given enzyme preparation with relation to a certain substrate and those studies where kinetics is employed as a tool in elucidating the mechanism of an enzymatic catalysis. In studies of the first type the experiments are arranged so as to yield information on *initial reaction rates* under standardized and well-controlled conditions. The disturbing effect of unfavorable temperatures, leading to irreversible inactivation, of unfavorable hydrogen-ion concentrations, causing reversible or irreversible diminution of reaction rates, of the depletion of substrate during the period of observation, and of similar masking factors is excluded as far as possible. In many instances it is possible to limit the observations to the linear portion of the reaction curve and thus to compare the activities of different enzyme solutions directly, that is, using simple proportionality factors and without recourse to mathematical equations. If, however, information is sought on the mechanism of an enzyme reaction, the process is studied under as great a variety of experimental conditions as possible. It is important to restrict the variation from experiment to experiment to *one* variable at a time so as not to impair the value of the information obtained. Thus, practically all observations on enzymatic reaction rates recorded in the literature prior to the introduction of buffer systems for the maintenance of a constant *p*H by Sørensen and Michaelis, *i. e.*, before 1909, are of doubtful significance since the *p*H changes, occurring almost invariably during the progress of the catalysis, superimposed their effect on those which it was actually desired to study. It must also be borne in mind that purely kinetic data, although comprehensive and obtained under ideal conditions, constitute only in isolated instances sufficient evidence for the reality of a postulated reaction mechanism. Independent evidence of a different kind, such as spectroscopic observations, is usually indispensable since the conclusions drawn from kinetic data and their mathematical interpretation are frequently ambiguous in that they are consistent with more than one type of mechanism. Thus, while the theory of Henri and of Michaelis and Menten was able to interpret the kinetic data obtained in the study of enzymatic reactions, satisfactorily in terms of the formation of intermediate enzyme-substrate and enzyme-inhibitor compounds, it was not until the existence of such complexes was demonstrated by direct spectroscopy many years later that their reality became an established fact.

(*a*) *Methods*

The ideal methods for kinetic studies are those which allow for a continuous observation of the progress of the reaction without disturbing the system by the withdrawal of samples at certain intervals. Such methods may involve the observation of pressure changes caused by the reaction (manometry), of volume changes (dilatometry), changes in conductivity, light absorption (spectrophotometry), optical rotation (polarimetry), electrical potentials (potentiometry), or any other changes in physical properties which are directly related to the enzymatic catalysis. In recent years, all of these techniques have been developed to a high level of refinement and precision, and many of the instruments required are available commercially. For a recent review of apparatus and methods which are applicable here, reference is made to the articles by Loofbourow[3] and by Müller.[4] If no such special apparatus is available or if the particular reaction under study does not lend itself to this type of observation, one is obliged to resort to the analysis of representative samples withdrawn from the reaction mixture at regular intervals. In that case it is important to "freeze" the composition of the sample so as to correspond exactly to the status of the system at the time of sampling. The main problem is, of course, to stop the further action of the enzyme on the substrate immediately after taking the sample, since the stability of the unreacted substrate is seldom a source of trouble. Perhaps the expedient most frequently used to terminate the enzyme action consists in shifting the *p*H to a region where the enzyme is completely inactive. To this end, the sample is transferred to a strong acid or alkali solution, depending on the nature of the substrate. No enzyme thus far studied is able to exert its activity at *p*H values below 1 or above 11. If for some reason such drastic changes in hydrogen-ion concentration must be avoided, the enzyme action may be stopped by rapid heating to the boiling point, by rapid freezing, or by the addition of some specific inhibitors, *e. g.*, cyanide in the case of enzymes representing iron porphyrin proteins or salicylaldoxime in the instance of copper protein enzymes.

---

[3] J. R. Loofbourow, *Rev. Modern Phys.*, **12**, 267 (1940).

[4] R. H. Müller, *Ind. Eng. Chem., Anal. Ed.*, **13**, 667 (1941).

In the analysis of the individual samples or in utilizing the readings obtained by some physical method during the progress of the enzyme reaction, it is not necessary to standardize the reagents or method in terms of *absolute* quantities of substrate which has been transformed under the influence of the catalyst if the *kinetics* of the reaction is being studied. It is usually sufficient to determine the reading on the buret or measuring instrument, corresponding to the total or initial concentration of the substrate, so that the readings obtained at any given interval during the reaction may be expressed in terms of fractions of the total substrate. If the substrate is present in great excess and if it is not appreciably depleted in the course of the experiment, or, more generally, if only the linear portion of an enzyme reaction is studied, it is often not even necessary to determine the value equivalent to the total substrate. For comparing the enzymatic activity of the unknown with a standard enzyme solution or for evaluating the *relative* activity of two unknown systems, it suffices to compare directly the slopes of the linear reaction curves. It is usually desirable to compare the *initial reaction rates*. If, for technical reasons, these cannot be determined directly, they may be obtained by extrapolating to zero reaction time from the values secured at certain intervals after the start of the experiment. This procedure is, of course, only valid if the known points on the reaction curve are not too far removed from the starting point and if these are situated on a straight line intersecting the ordinate; in other words, if there is no lag or induction period, followed by a gradual increase in velocity.

(*b*) *Determination of Order of Reaction*

We have to distinguish between the *absolute rate* of a given reaction and the velocity as a *function of time* (reaction order). These two chief characteristics of chemical processes are essentially independent of each other. A reaction of a low order may exhibit a high velocity and vice versa. If the reaction rate remains constant over the period of observation, we speak of a reaction of *zero order*. If the velocity decreases with time and approaches zero velocity in an asymptotic manner, the reaction may be of the *first, second,* or *third order*. In order to ascertain whether the process under study may be described in terms of a first or higher order reaction, the experimentally obtained values for substrate turnover as a function of time are inserted in the various equations developed for these reactions and the corresponding *reaction constants* are computed. If these "constants" are found to be actually constant throughout the period of observation, the process under study is stated to be of that reaction order the equation of which is fitted by the experimental data.

The general form of an equation, describing reaction velocities, is

$$dx/dt = k \cdot f(c_1, c_2 \ldots)$$

Here, dx/dt is the so-called time differential of the reaction, *i. e.*, the change in substrate concentration during a very small time interval, while k represents the velocity constant and c_1, c_2, etc., denote the concentrations of the various reactants. In the case of *elementary reactions*, *i. e.*, primary reactions of the smallest number of molecular species to which the over-all process can be reduced, $f(c)$, the concentration function, always represents a whole number power of c, it is of the first power for reactions of the first order, of the second power or else the product of two concentrations for second order reactions, and so on. Integration of the differential equations derived for the processes of different order yields the following expressions for the corresponding reaction constants which are suitable for performing the "test of constancy" mentioned above.

$$k_1 = \frac{1}{t} \ln \frac{c_A}{c_A - c_x} \quad \text{. constant of first order}$$

$$k_2 = \frac{1}{t \cdot (c_A - c_B)} \ln \frac{(c_A - c_x) \cdot c_B}{(c_B - c_x) \cdot c_A} \quad \text{. . .constant of second order}$$

$$k_3 = \frac{1}{2c_A{}^2 \cdot t} \cdot \left[\left(\frac{c_A}{c_A - c_x} \right)^2 - 1 \right] \quad \text{. . .constant of third order}$$

In these equations, c_A and c_B are the *initial* concentrations of the molecular species A and B, while $(c_A - c_x)$ and $(c_B - c_x)$ represent the concentrations of A and B at the time t at which the observations were made. The expressions for the higher order reaction constants become considerably simpler if the initial concentrations of the reactants are equal, *i. e.*, if the reactions take place between equimolecular amounts of the various substances. The expression for the second order constant is reduced in this case to the equation

$$k_2 = \frac{1}{c_A \cdot t} \left(\frac{c_A}{c_A - c_x} - 1 \right)$$

Since the integration of the general equation for third order reactions would yield a very complex expression, the formulation for k_3, as given above, is already based on the assumption that the initial concentration of all three reactants is the same ($c_A = c_B = c_C$).

Provided that the process under study is an elementary or primary reaction, the finding that its kinetics may be described by the equation for a first, second, or third order reaction allows one to draw conclusions concerning the number of molecular species involved in the process. This is the reason why the terms *monomolecular*, *bimolecular*, or *trimolecular* reaction are frequently used synonymously with the terms first, second, or third order reaction. In the instance, however, of more complex processes, such as catalyzed reactions, this usage is not justified. In complex reactions, the slowest link determines not only the speed, but also the apparent order of the over-all process. Strictly speaking, a monomolecular reaction is the transformation of a single molecular species into one or several product molecules. The classical example for monomolecular reactions is given by the spontaneous transmutations of radioactive substances. Many hydrolytic processes, however, *e. g.*, the inversion of sucrose, are satisfactorily described by the equation for a first order reaction, although we know that at least two molecular species, namely, the substrate and water molecules, are involved. Since water is usually present in such great excess that the only measurable change in concentration takes place with regard to the substrate molecules, the equation for a second order reaction is reduced to that for a first order process under these conditions. In such instances we speak frequently of "*pseudomonomolecular*" reactions. The same holds usually for the catalyst or enzyme promoting such reactions, since its concentration, over short time intervals at least, is not significantly changed during the catalysis.

Conversely, if the number of molecular species or the minimum number of molecules participating in a given reaction is known or deduced from the stoichiometry of the over-all reaction equation, it is not always possible to predict the order which will apply to the kinetics of that process. An interaction of molecules must be preceded by their collision. The number of successful collisions, leading to interaction, is a function of the concentration of molecular species concerned, of their energy content or state of activation, the geometrical conditions for the approach of the molecules to each other, and of the number of molecules which must collide at the same instant to bring about the reaction. Statistical considerations show that the probability for a trimolecular reaction is already quite small and that reactions of still higher order are so rare that events of this type can hardly be used in describing processes which occur with a measurable and sometimes even explosive rate. Experience, collected especially in the field of gas reactions, has shown, in full accord with this point of view, that over-all reactions of a seemingly high order can often be "dissected" into a sequence of elementary processes of lower order, usually into bimolecular reactions. The same holds undoubtedly also for reactions of biological interest such as enzymatic catalyses.

Frequently it is found that a reaction under study cannot be adequately described by the equations for the various orders of reaction mentioned above. If, for instance, the enzyme is irreversibly inactivated in the course of the catalysis, we will find that the velocity "constants" as calculated for a first order reaction are not constant with time but tend to decrease with the progress of the reaction. In such cases it is justifiable to extrapolate the values for the constant back to zero reaction time and use the k_1-value thus obtained to describe the activity of the enzyme preparation prior to the test reaction. A case in point is catalase which is very sensitive to its substrate, hydrogen peroxide, even at low temperatures. The course of catalase action is most closely described by the equation for a first order reaction, but $k_{\text{monomol.}}$ shows invariably a tendency to decrease with time. It is the accepted practice in this case to extrapolate the k-value back to the start of the reaction by a simple graphical procedure.

(*c*) *Influence of Temperature*

We have to distinguish between two kinds of temperature effects on enzymatic reactions. Within a certain range, between 0° C. and about 40 or 50° C., an increase in temperature will produce an increase in velocity of enzyme action which may be brought back to a former level upon cooling the system. This *reversible* effect of temperature is encountered in the study of chemical reactions in general, whether catalyzed or uncatalyzed. It is explained by assuming that at a given temperature a certain fraction of the molecules of a system has a higher than average energy content and, hence, chemical reactivity. Only these "energy-rich" molecules are capable of reaction at that temperature. Upon raising the temperature, some of the thermal energy introduced into the system is harnessed for the production of more reactive molecules. The mechanism of this thermal activation consists in the absorption and "storage" of heat energy in the various degrees of freedom of the molecule; in other words, it is transformed into vibrational or translational energy of the molecule. Such a molecule, in turn, is more likely to break down in smaller fragments (monomolecular reaction) or to interact with other particles upon collision (bi- and trimolecular processes) than the molecules endowed with only the average or even less than average energy content. As a general rule, chemical reactions are accelerated two- to threefold upon a rise in temperature of 10°. In this way they may be distinguished from certain physical processes, *e. g.*, diffusion or photochemical phenomena, which have a very small or negligible *temperature coefficient*. The latter, designated as Q_{10}, is defined as the ratio of the reaction velocities at $(T + 10)°$ and at $T°$, where T is the absolute temperature. As a further empirical rule, the Q_{10}-values found for enzymatic reactions are smaller than the temperature coefficients of the corresponding noncatalyzed or hydrogen ion catalyzed processes. Furthermore, the Q_{10}-values for enzyme reactions frequently decrease with increasing temperature. This is illustrated in Table 23, containing the Q_{10}-values for some enzymes. It will be noted from an inspection of the table that the change of Q_{10} with temperature is marked only in the instance of the hydrolytic enzymes mentioned. The theoretical significance of this phenomenon has been the subject of controversy and is still somewhat obscure.

From the reaction rates, as determined at two different temperatures, the so-called *critical increment* or *energy of activation* of the reaction within that temperature range may be calculated with the aid of the relationship

$$E = \frac{R \log \frac{k_2}{k_1}}{\frac{1}{T_1} - \frac{1}{T_2}}$$

where R is the gas constant, and k_1 and k_2 are the velocity constants of the process at the absolute temperatures T_1 and T_2, respectively. The critical increment is defined as that amount of energy which has to be supplied to a reaction system before the start of a "voluntary" (exothermal) process. In other words, it is a measure of the intensity of the impulse which is required to overcome the natural inertia of the system. The fact that the temperature coefficients, and hence the energies of activation of catalyzed reactions, are invariably smaller than those of the corresponding uncatalyzed processes forms the basis of a widely accepted concept of the function of a catalyst such as an enzyme. The catalyst induces the system to react rapidly by lowering the energy of activation, *i. e.*, by removing the obstacle in the path of the "metastable" reaction system. This decrease in the critical increment is accomplished by the intermediary combination of the enzyme with the substrate leading to the creation of an unstable product of changed energetic configuration. In the further course of the reaction the enzyme is restored to its free state and the energy initially supplied is "paid back" by the subsequent exothermal steps in the over-all catalysis. For a fuller discussion of the problem of enzymatic activation energies the reader is referred to a review article by Moelwyn-Hughes.[6]

Table 23

Temperature Coefficients of Some Enzyme Reactions[a]

Enzyme	Substrate	Temperature interval, ° C.	Temperature coefficient, Q_{10}
Pancreatic lipase	Ethyl butyrate	0–10	1.50
		10–20	1.34
		20–30	1.26
Liver lipase	Ethyl butyrate	0–10	1.72
		10–20	1.36
		20–30	1.10
Yeast maltase	Maltose	10–20	1.90
		20–30	1.44
		30–40	1.28
Malt amylase	Starch	20–30	1.96
		30–40	1.65
		40–50	1.43
Yeast dipeptidase	Glycyltyrosine	25–35	2.25
		35–45	1.56
Pancreatic erepsin	Glycyltyrosine	25–35	1.67
		35–45	1.60
Pepsin	Edestin	0–10	2.6
		10–20	2.0
		20–30	1.8
		30–40	1.6
		40–45	1.4
Trypsin	Casein	30–40	1.46
Urease	Urea	30–40	3.0
Fat catalase	Hydrogen peroxide	0–20	1.4
Root peroxidase	Leucomalachite green and hydrogen peroxide	5–15	2.0
		15–25	2.0
Succinodehydrogenase (*E. coli*)	Succinate	30–40	2.1
		40–50	2.1
		50–60	2.1

[a] These values were taken from Table XII in Haldane-Stern,[5] where the references to the original literature may be found.

---

[5] J. B. S. Haldane, *Enzymes*, Longmans, Green, London, 1930; J. B. S. Haldane and K. G. Stern, *Allgemeine Chemie der Enzyme*, Steinkopff, Dresden, 1932.

[6] E. A. Moelwyn-Hughes, *Ergeb. Enzymforsch.*, 2, 1 (1933).

Another type of effect of temperature on enzymes is essentially *irreversible*. In order to study this effect, the enzyme preparation is held at successively higher temperatures for stated intervals and each time brought back to a lower temperature at which it is stable and at which its activity is customarily determined. As a rule, *heat inactivation* of enzymes in solution is observed in the same temperature range (50 to 70° C.) in which proteins are denatured. This observation has greatly strengthened the view that enzymes belong to the class of proteins. But just as in the case of ordinary proteins, the heat lability of the various enzymes shows considerable variations from case to case and it is also affected by the presence or absence of protective substances. This explains why the thermostability of enzyme preparations decreases with progressive purification. Some crude enzyme solutions, *e. g.*, cathepsin extracts from spleen, may actually be boiled for brief periods without complete abolition of the enzymatic activity. Furthermore, it appears that water is required for the process of heat denaturation of enzymes (and proteins), since dry preparations may be heated to much higher temperatures than solutions without destruction of their activity. Another feature which enzymes share with proteins is the unusually large temperature coefficient of heat inactivation. The critical increment of this process may reach values greater than 100,000 cal. The *critical temperature* at which rapid thermal destruction of the enzyme occurs is usually well defined for a given preparation, but is influenced by the degree of purity of the material as mentioned above. The course of heat inactivation of enzymes follows frequently that of a monomolecular process. The "decay" of activity of pepsin particles as a function of temperature and hydrogen-ion concentration was recently studied in detail by Steinhardt.[7] The so-called *optimum temperature* of an enzyme action is the resultant of the reversible and irreversible temperature effects just given; since it depends on the experimental conditions, it is of little absolute significance, although its knowledge is useful from purely practical considerations. The heat inactivation of trypsin at acid *p*H is reversible (see below).

(*d*) *Influence of Hydrogen-Ion Concentration*

Just as in the case of the effect of temperature on enzymes, a variation in *p*H may influence their action in two ways, namely, reversibly and irreversibly. The sensitivity of enzymes to varying degrees of acidity and alkalinity differs considerably. Yeast saccharase, for instance, is rapidly inactivated in an irreversible manner at *p*H values below 3, while pepsin exhibits its maximum activity between 1.5 and 2.5. Crystalline trypsin may be boiled in dilute hydrochloric acid without losing its activity (Northrop[8]). It can be shown, however, that this is a case of reversible heat inactivation. Upon raising the temperature to the boiling point the activity decreases to zero and then it is spontaneously regained upon cooling the solution to room temperature. Inasmuch as crystalline trypsin represents a fairly homogeneous protein, this phenomenon is also an example of a perfectly reversible heat denaturation of a protein. Very few enzymes will withstand a *p*H as low as 1 or as high as 11 for any length of time. In the physiological range of hydrogen-ion concentrations, *i. e.*, between about *p*H 3 and 9, there is usually one fairly narrow region within which the activity of the enzyme under study will show a maximum of activity. The decrease of the latter at neighboring hydrogen-ion concentrations is not always symmetrical with regard to the *pH optimum*. As in the study of the effect of temperature on enzyme activity, two different sets of experiments may be devised to test the effect of *p*H. The reaction of the enzyme with its substrate may be allowed either to proceed at varying hydrogen-ion concentrations as they may be established by suitable buffer mixtures, or else the enzyme alone is exposed to a certain *p*H for a stated period and its activity toward the substrate is subsequently determined after shifting the *p*H back to its optimum value. The curves, as obtained by these two methods for tyramine oxidase, are shown in Fig. 13. According to the experiments forming the basis for curve *A*, the enzyme is not adversely affected by hydrogen-ion concentrations ranging from about *p*H 4.5 to 9. Beyond *p*H 9, first a minor and then a very great fall in enzyme activity is observed. From curve *B*, summarizing the activity of the enzyme at different hydrogen-ion concentrations, a *p*H optimum of about 10 is indicated. The marked asymmetry of the activity-*p*H curve together with the shape of curve *A* suggests that the true *p*H curve of the enzyme would be nearly symmetrical with respect to *p*H 10, were it not for the irreversible inactivation of the catalyst in the alkaline range. The corresponding curves for a number of hydrolytic enzymes, *e. g.*, carbohydrases, are usually found to be nearly symmetrical. This is made possible by the location of the *p*H optima in the neighborhood of *p*H 5 to 6 which is sufficiently far removed from the regions of

---

[7] J. Steinhardt, *Kgl. Danske Videnskab. Selskab. Math-fys. Medd.*, **14**, No. 11 (1937).

[8] J. H. Northrop, *Crystalline Enzymes.* Columbia Univ. Press, New York, 1939.

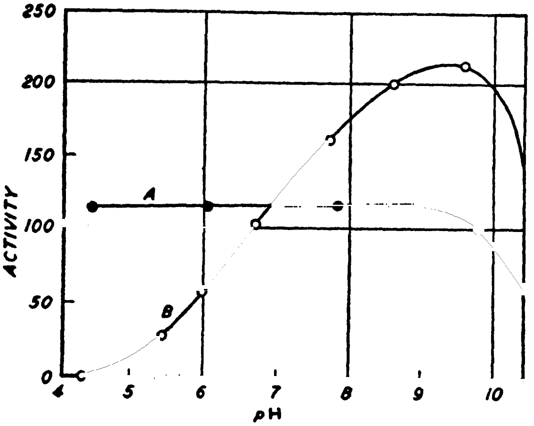


Fig. 13.—The influence of hydrogen-ion concentration on the activity of the tyramine oxidase system.[9] *A:* activity of enzyme at *p*H 7.3 after being subjected for 5 min. to various hydrogen-ion concentrations. *B:* activity of enzyme at different hydrogen-ion concentrations. The apparent optimum is clearly due to irreversible inactivation.

---

[9] M. L. C. Hare, *Biochem. J.*, 22, 968 (1928).

extreme acidity or alkalinity to permit the true *p*H-activity relationships of the enzyme to become manifest. The relation between *p*H and enzyme activity is affected by many factors, such as temperature, substrate concentration, purity of the enzyme preparation, nature and ionic strength of the buffer system employed, etc. The *p*H optima of a number of enzymes, hydrolytic as well as oxidizing, are listed in Table 24.

Michaelis and Davidsohn[10] have drawn attention to the fact that the activity-*p*H curves of certain enzymes, such as saccharase, resemble the dissociation rest curves of amphoteric electrolytes, such as proteins. These curves are constructed by plotting the fraction of molecules remaining in uncharged condition, against *p*H. The peak of such a curve represents, by definition, the isoelectric point of the ampholyte. While in some instances the isoelectric point of an enzyme, such as fairly crude yeast saccharase, as determined from electrophoretic experiments, coincides with its *p*H optimum, this is not invariably the case. This fact has led to the hypothesis that the *p*H optimum is a function of the properties of an intermediary enzyme-substrate compound rather than of the free enzyme. Further complications arise when the substrate, too, is an amphoteric electrolyte. Thus, in the instance of proteolytic enzymes, it appears that the charge on the proteins, serving as substrates, is even more important than the charge on the enzymes. Pepsin attacks only cationic protein particles, while trypsin reacts preferentially with protein anions. The class of catheptic enzymes, which includes also papain, exhibits the most marked activity on isoelectric proteins. In conclusion, it may be pointed out that the *p*H optimum of an enzyme does not invariably coincide with the hydrogen-ion concentration at which it exhibits maximum stability. While this is true for yeast saccharase, pepsin is most stable at *p*H 4 whereas its *p*H optimum is situated near 2.

(*e*) *Effect of Enzyme Concentration*

As a rule, the velocity of an enzymatic catalysis varies with the enzyme concentration. Unless some other factor such as the substrate concentration limits the rate of reaction, the velocity usually increases in a linear fashion with an increase in enzyme concentration. As has been pointed out above, this fact is frequently utilized in determining the relative enzyme concentration by comparing the initial reaction rates as produced by two different enzyme preparations. In his experiments with yeast saccharase, Hudson[11] varied the concentration of the enzyme over a range of 1 to 8 and then determined the percentages of sucrose inverted at various intervals under optimal conditions of temperature and *p*H. He found, at three different levels of original sucrose concentration, that for a given initial sugar concentration the product of enzyme concentration and time was the same. In other words, the fraction of sugar hydrolyzed by two enzyme units in 15 min. was the same as that split by one enzyme unit in 30 min. Many similar observations have been recorded in the literature. A number of apparent exceptions of this rule have been traced to a shift in *p*H,

---

[10] L. Michaelis and H. Davidsohn, *Biochem. Z.*, **35**, 386 (1911).

[11] C. S. Hudson, *J. Am. Chem. Soc.*, **30**, 1160 (1908).

TABLE 24

*p*H OPTIMA AND MICHAELIS CONSTANTS OF ENZYMES

Enzyme	Source	Substrate	*p*H optimum	K_m	Notes
A. Hydrolytic Enzymes					
Phosphatase	Mammalian tissue	Monophosphoric esters	9.0–9.2	....	....
		Diphosphonic esters	~7.8	....	
	Bone	Glycerophosphate	.....	<0.003 *m*	
	Aspergillus	Glycerophosphate	5.5	0.09 *m*	
	Malt	Phytin	5.6	....	
Lipase	Stomach (man)	Tributyrin	6	....	
	Pancreas	Ethyl butyrate	7	>0.03 *m*	In phosphate buffer
	Liver	Ethyl butyrate	8.3	....	
	Liver (man)	Methyl butyrate	.....	0.0085 *m*	
Maltase	Human intestine	Maltose	6.1	....	....
	Yeast	Maltose	6.6	0.12–0.3 *m*	....
Saccharase	Mammalian intestine	Sucrose	6.2	0.02 *m*	
	Yeast	Sucrose	4.6–5.0	0.016–0.04 *m*	
Amylase	Saliva and pancreas	Starch	6.0–7.0	0.08–0.25%	*p*H optimum varies with anions present
	Mammalian intestine	Starch	~7	....	
Urease	Soybean	Urea	7.2–7.9	~0.025 *m*	Values of constants vary with substrate concentration
Pepsin	Stomach	Various proteins	1.5–2.5	4.5%	K_m det. with ovalbumin
Trypsin	Pancreas	Various proteins	8–11	0.75–2%	K_m det. with casein
Papain	Carica papaya	Various proteins and peptones	5–7.2	....	Activated by HCN or H_2S
Cathepsin	Spleen and liver	Various proteins	3–5	....	Most active against isoelectric proteins
Polypeptidase	Intestine	Simplest dipeptides	~7	....	....
Dipeptidase	Intestine	Simple dipeptides	7.3–8.1	0.02–0.07 *m*	K_m for glycylleucine

TABLE 24 (*Continued*)

*p*H OPTIMA AND MICHAELIS CONSTANTS OF ENZYMES

Enzyme	Source	Substrate	*p*H optimum	K_m	Notes
		B. Desmolytic Enzymes			
Zymase complex (alcoholic fermentation system)	Yeast	Glucose	4.5–6.5 5.5–8	0.006 *m*	Rate of CO_2-formation glucose breakdown
Carboxylase	Yeast	α-Keto acids	4.8	0.01 *m*	K_m for pyruvic acid
Catalase	Liver	H_2O_2	6.3–9.5	0.025 *m*	....
Peroxidase	Roots	H_2O_2 + guiacol	~5	....	According to Theorell
		H_2O_2 + leucomalachite green	...	6.10^{-6} *m*	Mixture of two enzymes
Lactic dehydrogenase	*E. coli*	Lactate	9	0.005 *m*	
Succino-oxidase	Muscle	Succinate O_2 as acceptor	9	0.001 *m*	Complex system contg. cytochrome oxidase, cytochrome c_1, and succinic dehydrogenase
Succinic dehydrogenase	*E. coli*	MB as acceptor	9	0.002 *m*	....
Xanthine oxidase (Schardinger enzyme)	Milk	Xanthine, hypoxanthine acetaldehyde	~7	$<3.10^{-5}$ *m*	*p*H optimum not well defined
	Milk			>1 *m*	
Citric dehydrogenase	Muscle	Citrate	...	0.0005 *m*	....
Glutamic dehydrogenase	Muscle	Glutamate	...	0.0005 *m*	....

NOTE: The above data were taken from Tables II and V of Haldane-Stern.[1] They refer mostly to enzymes in a relatively low degree of purity. Inasmuch as the food technologist is likely to deal with the action of enzymes in their natural environment such as foodstuffs (yeast, etc.) and secretions (milk, etc.) these data are given here rather than those obtained more recently on highly purified or crystalline enzyme preparations.

caused in the system by the introduction of large amounts of impure enzyme preparations. A few genuine exceptions, however, have been found. The case of pepsin is the best known. Here, the velocity of protein hydrolysis is approximately proportional to the square root of the enzyme concentration. This relationship, called the "Schütz rule," has also been found by Arrhenius to hold for the tryptic digestion of gelatin and casein. Over a considerable range the amount of protein hydrolyzed could be expressed by the formula $K\sqrt{et}$ where e is the enzyme concentration and t is the time. Later it was shown by Northrop that highly purified pepsin no longer follows Schütz's rule but exhibits a nearly linear relationship between enzyme concentration and activity. If, however, peptic digestion products (peptones) are added to the system, a curve similar to that obtained with crude enzyme preparations results. The phenomenon has been explained by the affinity of the enzymatic split products for the enzyme, *i. e.*, for their competition with the substrate for the same groups in the enzyme molecule.

Another instance, where there exists a lack of proportionality between enzyme concentration and reaction rate, is that of amylase. Amylase will not hydrolyze starch completely, a certain amount of difficultly hydrolyzable dextrin being produced along with the main reaction product, namely, maltose. These partial degradation products, however, are rendered sensitive to the action of the enzyme by the addition of certain protein-split products, called *complement*, which appear to combine with the dextrin. This somewhat mysterious factor and its presence in varying amounts in amylase preparations are deemed to be responsible for the anomalous phenomena observed in the study of amylase action.

(*f*) *Influence of Substrate Concentration*

The variable represented by the concentration of substrate is of considerable importance and interest, both from a practical and a theoretical point of view. If we speak of substrates, we do not, as a rule, have in mind the *water*, although, besides being the solvent for all enzymatic catalyses, water is also a chemical reactant in hydrolytic processes. Ordinarily, however, it is present in such great excess that it does not constitute a limiting factor in the kinetics of such reactions and, consequently, does not figure in the kinetic equations. Moreover, the affinity of hydrolytic enzymes for water molecules appears to be very small, thereby avoiding any competition with the main substrate for active groups in the catalyst. The situation is different, however, when we are dealing with highly concentrated solutions. Thus, Nelson and Schubert[12] observed a maximal velocity of sucrose inversion by yeast saccharase at sugar concentrations of about 5%. The linear decrease in reaction rate resulting from an increase in substrate concentration from 10 to 70% could be shown to be caused largely by the simultaneous decrease in water concentration. This was demonstrated by comparing the effect of higher sugar concentrations with that obtained by adding varying amounts of alcohol while the substrate concentration was kept unchanged.

---

[12] J. M. Nelson and M. P. Schubert, *J. Am. Chem. Soc.*, 50, 2188 (1928).

Let us now examine the influence on the reaction velocity by variations in substrate concentration which do not, at the same time, cause an appreciable decrease in the water concentration. If this is done gradually, beginning with very low substrate concentrations, and if all other variables are properly controlled, the initial rate of the catalysis increases at first proportionally to the substrate concentration. At a certain substrate concentration, a maximum rate is observed which may either be maintained over a considerable range of still higher substrate concentrations or may show a decline in the presence of excess substrate. Such a decline often occurs under conditions where the water concentration cannot be a limiting factor. This decrease in rate at high substrate concentrations may be reversible or irreversible in nature. Sometimes as in the case of catalase, it has a reversible as well as an irreversible component and, thereby, renders a theoretical interpretation of this part of the curve difficult. If, according to the proposal of Michaelis and Menten[13] the negative logarithm of substrate concentration (as the abscissa) is plotted against the reaction rate in some arbitrary units (as the ordinate), an S-shaped curve, called the *activity-P_S curve*, is obtained for the range of substrate concentrations from zero to that at which a velocity maximum is located (see Fig. 14).

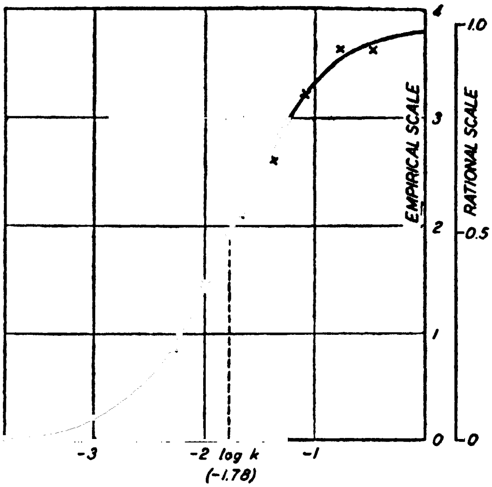


Fig. 14.—Activity-P_S curve of saccharase. *Abscissa:* logarithms of initial sucrose concentrations. *Ordinate:* initial reaction rates. (From Michaelis and Menten.[13])

If now a rational measure is introduced for the ordinate, designating the ordinate value of the maximum as 1.0, then the parameter of the curve, corresponding to a distance of 0.5 on the ordinate, yields a point on the abscissa, designated as log *k*. This point is the negative logarithm of that substrate concentration at which the reaction will proceed with one-half the maximum rate. The theoretical significance of this value, according to Michaelis, lies in the fact that it represents the dissociation constant of the enzyme-substrate compound which forms an essential intermediate in the catalysis. The value for the substrate concentration as obtained from log k, expressed in moles per liter, has been termed the *Michaelis constant*, K_m, and is a characteristic constant for any given enzyme. A number of Michaelis constants of different enzymes will be found in Table 24. Since the velocity maximum is usually reached in an asymptotic manner, the substrate concentrations corresponding to the half-maximum reaction rate is better defined than that obtaining at the maximum itself. The Michaelis constants, *i. e.*, the substrate concentration corresponding to half saturation of the enzyme with substrate, are usually of a different order in the case of oxidative than in that of hydrolytic enzymes, as may be seen from an inspection of the table. The much lower values for the Michaelis constants of oxidative enzymes indicate that this type of enzymes has a much higher affinity for their substrates than is the case for hydrolytic enzymes. Catalase occupies an intermediary position in this respect. The reciprocal value of the Michaelis constant, $K_M = (1/K_m)$, may be considered to be a measure of the affinity of the enzyme to its substrate and is therefore called the "affinity constant." Thus, the Michaelis constant, K_m, for saccharase with respect to sucrose is 0.0167 (or $^1/_{60}$), while the affinity constant, K_M, for the same combination is 60.

---

[13] L. Michaelis and M. L. Menten, *Biochem. Z.*, **49**, 333 (1913).

As has already been mentioned, the activity-P_S curve of some enzymes shows a decline in the range of high substrate concentrations, beyond the point corresponding to maximum reaction rate. The descending branch of the curve is usually fairly symmetrical with regard to the ascending branch, so that a dumbbell-shaped curve results. Examples of this type are catalase and lipase. It has been assumed that this phenomenon is due to the formation of *stable* enzyme-substrate complexes of higher order, *i. e.*, containing more than one substrate molecule per enzyme molecule. The parameter of the descending part of the curve has consequently been called the "second Michaelis constant" and has been interpreted as the dissociation constant of the stabilized enzyme-substrate compound.

For a recent discussion of the kinetics of hydrolytic enzymes and their bearing on methods for measuring enzyme activity, the reader is referred to a paper by van Slyke.[14]

(g) *Activation and Inhibition of Enzymes*

The activity of enzymes is not only a function of the hydrogen-ion concentration, temperature, substrate concentration and similar, well-defined and readily controlled physical-chemical factors. If this were the case, one might be able to construct a nomogram of the type employed by L. J. Henderson in describing and defining the state of blood under varied conditions as a unique function of a finite number of variables. The only instance where such an ideal state of things is approached to some degree in enzyme chemistry is the one where a pure, *i. e.*, homogeneous, enzyme preparation is available. The number, however, of unquestionably homogeneous enzyme preparations thus far described is very small indeed, in spite of the fact that an increasing series of enzymes has been prepared in the form of crystalline proteins. It is increasingly recognized that crystalline form in the case of a protein is no guarantee that we are dealing with a pure substance, even if the material has been repeatedly recrystallized and has retained a constant level of activity throughout these operations. It would appear that other techniques such as electrophoresis under optical control and differential ultracentrifugation are fundamentally better suited for the ultimate separation of an enzyme from associated substances than salting-out methods, fractionation by organic solvents, or isoelectric precipitation. In less highly purified enzyme preparations there are almost invariably present trace constituents which exert an activating or an inhibiting effect on the activity of the catalyst. The problem is complicated by the fact that the enzyme itself is usually present only in very minute amounts so that we are dealing here with the general problem of the interaction of at least two components of a reaction mixture at extreme dilutions. This situation frequently makes it extremely difficult, if not impossible, to ascertain the mechanism of action of unknown promoters or poisons of an enzymatic catalysis. For reasons of practical convenience an attempt has been made to label by different designations the different types of activating and inhibiting substances. The former have been subdivided into *coenzymes*, *activators*, *kinases*, and *complements;* the latter into *specific* and *unspecific* or in *competitive* and *noncompetitive inhibitors*.

---

[14] D. D. van Slyke in *Advances in Enzymology*, Vol. II. Interscience, New York, 1942, p. 33.

Haldane[15] has defined a *coenzyme* as a "heat-stable, crystalloidal organic substance of fairly high specificity associated with an enzyme in nature." As a consequence of the recent work on pyridine, alloxazine, and thiamine coenzymes, a strong tendency has arisen to identify a coenzyme with the *prosthetic group* of the enzyme concerned, *i. e.*, as the dissociable, nonprotein component of the enzyme itself, representing the active group of the biocatalyst. This group of activators will therefore be discussed in the second part of this article (page 219). The term *kinase* was coined to convey the view that such substances as enterokinase or thrombokinase act as catalysts in the transformation of inactive precursors into the enzymes trypsin and thrombin, respectively. The nature of the interaction between trypsinogen and enterokinase, however, still remains to be elucidated. The problem is complicated by our scant knowledge of the kinase which has not yet been isolated in pure form. In recent years, hydrogen cyanide and hydrogen sulfide have been considered by some workers to represent kinases for the proteinases of the third group or catheptic enzymes. The fact that papain, cathepsin, and other members of this group are activated strongly by these substances has been interpreted successively as a result of the formation of active enzyme-HCN or -H_2S compounds, endowed with a new type of specificity (Mendel and Blood, Willstätter, *et al.*), the removal of heavy metal inhibitors by complex formation (Krebs), and the reduction of the inactive disulfide (S-S) form of these enzymes into the active sulfhydryl (SH-) form (Bersin, Hellerman, and others). Very recently, Bergmann and Fruton[16] favor once again the original hypothesis of Mendel and of Willstätter, namely, the formation of papain-activator complexes, with the modification that the latter combination is preceded by the reduction of an inactive α-proenzyme to an equally inactive β-proenzyme.

---

[15] J. B. S. Haldane, *Enzymes*, Longmans, Green, London, 1930; J. B. S. Haldane and K. G. Stern, *Allgemeine Chemie der Enzyme*, Steinkopff, Dresden, 1932.

The *complement* of amylases, which according to Pringsheim renders certain dextrin fractions sensitive to the action of these enzymes and in this way extends the range of specificity to new substrates, has already been briefly mentioned.

The term *activator* is generally applied to substances which appear to be necessary or beneficial for the action of an enzyme but the mode of action of which is still unknown. Into this category falls the activation of certain proteolytic enzymes by some heavy metals and metal complexes (Michaelis and Stern[17]), the effect of manganese salts on arginase (Hellermann[18]), the action of chloride and other anions on animal amylases, of magnesium and manganese on certain phases of alcoholic and lactic acid fermentation, and many other similar observations.

At this point a special class of substances should be mentioned, the presence of which, in small amounts, is necessary for the attainment of measurable rates in certain complex enzymatic reaction systems. We refer here to the role of adenylic acid or acetaldehyde in alcoholic fermentation or of ornithine in the urea production by liver slices *in vitro*. These substances have frequently been designated as catalysts for these processes, because their addition is required to overcome induction periods, to establish measurable reaction rates, and because they may be recovered unchanged at the conclusion of the reaction. While it is true that these are also criteria of catalysts, these compounds are actually *indispensable intermediates or reactants* rather than true catalysts. Thus, ornithine and citrulline are intermediates in the cycle, operative in urea formation, which is actually catalyzed by enzymes such as arginase, etc. The same is true for adenylic acid which is an essential intermediate in the phosphate transfers occurring in fermentation. Here again, special enzymes catalyze the phosphorylations and transphosphorylations involved. Acetaldehyde, which shortens the induction period of yeast fermentation, acts as the acceptor for the hydrogen atoms of reduced cozymase. But the reaction between the two is possible only in the presence of the activating protein of acetaldehyde reductase. Other instances of this kind are the "catalytic" effects of small amounts of succinic and fumaric acids on tissue respiration, of methylene blue and pyocyanine on the respiration of red blood cells, bacteria, and marine eggs, etc.

The reason why traces of starch are required for the resynthesis of glycogen or starch from Cori ester (glucose-*l*-phosphate) by phosphorylase is still obscure. One of the explanations which have been suggested is that the enzyme requires a colloidal surface for its synthetic action. But this is essentially an *ad hoc* hypothesis.

---

[16] M. Bergmann and J. S. Fruton in *Advances in Enzymology*, Vol. I. Interscience, New York, 1941, p. 63.

[17] L. Michaelis and K. G. Stern, *Biochem. Z.*, **240**, 192 (1931).

[18] L. Hellermann and M. E. Perkins, *J. Biol. Chem.*, **112**, 175 (1935).

The variety of agents and mechanisms by which enzymes may be *inhibited* is just as great if not greater than that by which they can be *activated*. The inhibitors may be classified as specific and unspecific, or reversible and irreversible, or as competitive and noncompetitive in their action. The last-named classification has the advantage that its establishment for a given case frequently has not only shed light on the mode of action of the inhibitors studied, but also on the chemical nature of the enzyme under investigation. Thus, the experiments of Warburg which demonstrated the competition of carbon monoxide with oxygen for the oxygen transferring enzyme of respiration and thereby explained the nature of CO-poisoning of respiration eventually led to the elucidation of the structure of the catalyst as a pheohemin protein.[19]

Competitive inhibitors compete with the substrate for the active group of the enzyme or with the enzyme for the substrate, while *noncompetitive inhibitors* either combine with a different grouping of the enzyme molecule than does the substrate or they interfere with the breakdown of the enzyme-substrate compound, *e. g.*, by breaking reaction chains. It is often possible to differentiate between these two types of inhibitors by examining their effect on the activity-P_S curve of the enzyme (see page 201). The method consists in maintaining the concentration of the enzyme and of the inhibitor at a constant level while studying the relationship between substrate concentration and reaction velocity. If the percentage inhibition remains the same over a wide range of different substrate concentration, we are dealing with an inhibition of the noncompetitive kind. If, on the other hand, the extent of inhibition of the enzyme is decreased by increasing the substrate concentration, the phenomenon is of the competitive type. In the first case the Michaelis constant of the enzyme is not affected while in the second instance there is an apparent shift in the magnitude of the Michaelis constant in the direction of a decrease in affinity.

Haldane[20] lists the following types of enzyme poisons.

1. Substances combining with the enzyme as an acid, *e. g.*, protein precipitants.
2. Substances combining with the enzyme as a base, *e. g.*, heavy metals.
3. Oxidizing agents.
4. Amine reagents, *e. g.*, formaldehyde, nitrous acid.
5. Aldehyde reagents.
6. HCN, H_2S, $H_4P_2O_7$, NO.
7. Surface-active substances, *e. g.*, substituted urethanes, and ions influencing colloidal behavior.
8. Other crystalloidal substances whose mode of action is obscure.
9. Colloidal and semicolloidal inhibitors, including immune bodies.

For an example of an experimental study of a variety of factors interfering with the activity of an enzyme, reference is made to an investigation of the various modes of inhibition of liver catalase (Stern[21]).

---

[19] O. Warburg, *Angew. Chem.*, **45**, 1 (1932).

[20] J. B. S. Haldane, *Enzymes*, Longmans, Green, London, 1930; J. B. S. Haldane and K. G. Stern, *Allgemeine Chemie der Enzyme*, Steinkopff, Dresden, 1932.

[21] K. G. Stern, *Z. physiol. Chem.*, **209**, 176 (1932).

TABLE 25

SOME INHIBITORS OF ENZYME ACTIONS

Inhibitor	Effective concentration	Enzyme inhibited	Mechanism of inhibition	Type of action	Remarks
Cyanide	10^{-3} *M*	Cytochrome oxidase	Combines with Fe··· of enzyme	Reversible, noncompetitive	**Inhibits respiration**
	10^{-2} *M*	Component of fermentation complex	Unknown	Reversible	**Inhibits alcoholic fermentation**
	10^{-2} *M*	Xanthine oxidase	Cyanhydrin formation (?)	Slow, irreversible	**Substrate protects enzyme against HCN**
Carbon monoxide	70 vol. %	Cytochrome oxidase	Forms Fe·· (CO) complex	Reversible, light-sensitive, competitive	**Extent of inhibition depends on CO/O$_2$ ratio**
	30 vol. %	Pasteur enzyme	Forms Fe·· (CO) complex	Reversible, light-sensitive, competitive	
Narcotics (valeronitrile, phenylurethane, etc.)	Varies with narcotic	Dehydrogenases	Surface adsorption	Reversible	**Effect depends on adsorption constant**
Cystine and S-S glutathione	......	Succinic dehydrogenase	Oxidation of SH- to S-S groups	Slowly reversible	
Potassium bromate	....	Seed proteinase	Oxidation	Irreversible	**Improves baking with wheat flour**
Phenylhydrazine	10^{-3} *M*	HCN-papain	Combines with carbonyl groups of enzyme (?)	Irreversible	**Prevents splitting of certain synthetic substrates**
Fluoride	10^{-3} *M*	Phosphatases	Unknown	Irreversible	**Inhibits fermentation**
Malonic acid **Maleic acid** **Oxaloacetic acid**	10^{-2} *M*	Succinic dehydrogenase	Combine with active group of enzyme	Competitive, reversible	**Inhibit respiration**
Dinitrophenols and trichlorophenol	10^{-3} *M*	Flavoproteins (*d*-amino acid oxidase, heart flavoprotein)	Unknown	Competitive	**Inhibit fraction of respiration, cell division, Pasteur effect**
Eserine (physostigmine)	10^{-3} mg./cc.	Choline esterase	Combines with enzyme	Reversible	
Methylisocyanide	10^{-2} *M*	Pasteur enzyme	Combines with enzyme	Reversible	**Specific for Pasteur Reaction**

Some inhibitors of enzyme reactions are listed in Table 25. The data are merely given by way of example and could be amplified almost *ad libitum*. For details reference is made to Oppenheimer's handbook on enzymes and to the recently published supplement.[22] The action of substances interfering with oxidation-reduction processes is also discussed in some detail in a recent monograph on *Biological Oxidation.*[23]

Three types of competitive inhibitors deserve special mention. In many instances the *split products*, resulting from the action of an enzyme on a substrate, possess some affinity for the active groups of the catalysts. As they accumulate, therefore, the reaction gradually slows down and eventually comes to a standstill. This point does not necessarily represent an equilibrium between the forward and back reaction (or synthesis). Thus, proteinases are often inhibited by protein-split products under conditions where no resynthesis of proteins or protein-like materials, such as plasteins, occurs. That we are not dealing here with a true equilibrium can be shown, furthermore, by the fact that addition of fresh enzyme will bring about a further decomposition of substrate. Inasmuch as enzymes, being true catalysts, do not affect the position of the equilibrium point between forward and back reaction, it is clear that the slowing down of the catalysis was due to the competitive inhibition of the enzyme, added originally, by the product molecules. An irreversible inactivation of the catalyst by side reactions, however, may be responsible for a certain fraction of the over-all effect observed.

A somewhat related phenomenon is the inhibition of enzymes by substances which, in their chemical configuration, bear a close resemblance to the substrate or substrates of the enzyme. Some of these compounds are actually attacked by the enzyme, but at a much smaller rate than the chief substrate. A case in point is the competitive inhibition of the decomposition of hydrogen peroxide by catalase by ethyl hydrogen peroxide[21] which was recently shown to combine with the enzyme to form a spectroscopically well-defined enzyme-substrate complex which subsequently breaks down to regenerate the free enzyme and lower split products.[24] Other substances form *stable* combinations with an enzyme, of the kind analogous to enzyme-substrate compounds and, in this way, prevent the enzyme from reacting with the usual substrate. Such inhibitors might be termed "*pseudo-substrates.*" Examples are γ-quinoline pyruvic acid which prevents carboxylase from attacking pyruvic acid (Neuberg and Minard[25]) and maleic acid which interferes with the succinic-fumaric acid equilibrium as catalyzed by succinic dehydrogenase (Quastel). It might be argued that such apparently stable enzyme-pseudosubstrate compounds break down at a finite though hardly measurable rate which would permit us to treat this type of competitive inhibition as a special aspect of the larger problem of absolute and relative specificity.

The third kind of competitive inhibitors are the *anti-enzymes*, proteins of the type of immune bodies, which may be produced by injecting the corresponding enzyme into the blood stream of suitable animals. Anticatalase and antipepsin, which are found in the serum of rabbits, previously "immunized" with catalase and pepsin, respectively, are examples of such anti-enzymes. Their inhibitory action on the enzymes is undoubtedly based on their combination with the protein component of the catalysts to form combinations which are analogous to antigen-antibody complexes. The specificity of such anti-enzymes is very pronounced. Thus, the anti-enzyme, produced by immunizing the animal with chicken pepsin, will not inactivate swine pepsin (Herriott).

---

[22] C. Oppenheimer and R. Kuhn, *Die Fermente und ihre Wirkungen*, Thieme, Leipzig, 1925; C. Oppenheimer, *Supplement*, Junk, Amsterdam, 1936–1939.

[23] C. Oppenheimer and K. G. Stern, *Biological Oxidation*. Junk, Amsterdam, 1939.

[24] K. G. Stern, *J. Biol. Chem.*, **114**, 483 (1936); *Enzymologia*, **4**, 145 (1937).

[25] C. Neuberg and G. Minard, *Enzymologia*, **1**, 161 (1936).

(*h*) *Enzyme Units*

The definition of enzyme units is just as arbitrary and empirical as that of hormone or vitamin units. It represents that amount of an enzyme which, under specified conditions, will bring about a certain turnover of substrate. Its sole purpose and justification are that it makes it possible to compare the activity of different enzyme preparations under controlled conditions and reduce the results to a common denominator. The activity of an enzyme preparation, when referred to the content in solids or in nitrogen per unit volume, is a yardstick of its purity. A constancy of this quotient, if it is maintained throughout a series of consecutive preparative operations, has frequently been cited in support of the assumption that the enzyme had been isolated in chemically pure form. It has been demonstrated, however, that this conclusion is not legitimate, inasmuch as even recrystallized enzyme preparations may contain appreciable amounts of inert material, usually of protein nature and capable of forming mixed crystals or solid solutions with the enzyme. The purity, that is, the homogeneity, of enzyme preparations is best ascertained by the methods of modern protein chemistry, *e. g.*, electrophoresis, ultracentrifugation, solubility curves, etc., in conjunction with studies of their specific activity as displayed toward standard substrates under closely defined conditions.

Some of the enzyme units in current use will now be mentioned. Many of these were proposed by R. Willstätter and his school.

Saccharase Unit is that amount of enzyme which, when added to a solution of 4 g. of sucrose in 25 ml. of a 1% secondary sodium phosphate solution, reduces its optical rotation to zero within 1 min. at 15.5°. Prior to the experiment, the mutarotation of the α-glucose must be brought about by adding alkali. The decrease in optical activity of the substrate corresponds to a cleavage by the enzyme to an extent of 75.5% (Willstätter and Kuhn).

Lipase Unit is that amount of enzyme which, at 30° C. and within 1 hr., will hydrolyze 24% of 2.5 g. of olive oil of a saponification value of 185.5 in a system containing 2 ml. N ammonia-ammonium chloride buffer of pH 8.8, 10 mg. calcium chloride, and 15 mg. egg albumin in a total volume of 13 ml. (Willstätter, Waldschmidt-Leitz, and Memmen).

Phosphatase Unit is defined as the quantity of enzyme which, at pH 9 and 35° C. and in the presence of 0.001 to 0.006 M magnesium salt, will liberate the amount of phosphate corresponding to 0.1 mg. P from β-glycerophosphate within 1 hr. (Albers).

Urease is that amount of enzyme which produces 1 mg. ammonia-nitrogen from urea at 20° C. and *p*H 7.0 during the course of 5 min. Sumner's crystalline urease contains 133,000 units per gram.

Peroxidase.—The activity of peroxidase is expressed in terms of the "*Purpurogallin Number*" (P. Z.), which is defined as the number of milligrams of purpurogallin produced by 1 mg. of an enzyme preparation in 5 min. and at 20° C. in a system containing 5 g. of pyrogallol and 50 mg. hydrogen peroxide in a volume of 2 liters (Willstätter and Pollinger). The most active peroxidase thus far prepared was derived by Willstätter from horse-radish and had a purpurogallin number of over 3000. Another standard substrate, proposed for this enzyme, is leucomalachite green (Willstätter).

Catalase.—The activity of catalase preparations is usually expressed in terms of $k_{\text{monomol.}}$, *i. e.*, of the velocity constant of the first order, as calculated from the rate of decomposition of a 0.01 *N* hydrogen peroxide solution in phosphate buffer of *p*H 6.8 and at 0° C. Inasmuch as k tends to decrease with time, the value as extrapolated for reaction time zero is usually given. The purity of a catalase preparation is given by Kat. f., or catalase capability, which is defined as the ratio $k_{\text{monomol.}}$/g. of dry substance in 50 ml. reaction mixture. Horse liver catalase preparations, showing an activity as high as $k = 161{,}000$, have been obtained by a combination of chemical purification procedures and ultracentrifugal sedimentation (Stern and Wyckoff). Crystalline beef liver catalase has a Kat. f. of 25,000 to 26,000 (Sumner and Dounce). By ammonium sulfate precipitation, Agner has obtained amorphous horse liver catalase preparations of a Kat. f. of 55,000.

Proteinases.—In defining proteinase units, a variety of techniques (titration of liberated NH_2 and COOH groups, viscosimetry, colorimetry, nephelometry, etc.) and of substrates (albumin, casein, gelatin, edestin, etc.) have been employed by Waldschmidt-Leitz, Northrop, and other workers. In recent years, Northrop and his associates have advocated the use of hemoglobin as the standard substrate for proteinases. The colorimetric determination in this case is based on the blue color developed by the tyrosine- and tryptophane-containing split products with a phenol reagent. Denatured hemoglobin is digested by the enzymes under standard conditions, the undigested protein is precipitated with trichloroacetic acid, and the amount of unprecipitable split products is estimated colorimetrically. One proteinase unit is defined as that amount of enzyme which attacks hemoglobin, under the standard conditions, at a rate resulting in the liberation per minute of an amount of split products yielding a color intensity with the phenol reagent corresponding to one milliequivalent of tyrosine. The specific activity is defined as the activity per mg. of enzyme nitrogen. The activity of pepsin, trypsin, and papain may be assayed by this technique (see Northrop[26]).

---

[26] J. H. Northrop, *Crystalline Enzymes*. Columbia Univ. Press, New York, 1939.

2. Specificity

The problem of enzymatic specificity is of great practical and theoretical importance. By specificity we mean the relationship between enzyme action and chemical structure of the substrates or, rather, potential substrates. We distinguish between *absolute* and *relative* specificity. Enzymes endowed with absolute specificity limit their action to a single substrate or to a single type of chemical linkage. The instances where only one compound is attacked by an enzyme appear to be rather rare. Cytochrome oxidase, which catalyzes the oxidation of ferrocytochrome c by oxygen, is one example. In most cases where an enzyme was originally believed to possess absolute specificity toward a single substrate, it was later found to attack closely related substances, although at a much slower rate. Thus, catalase, which was long considered to be active uniquely against hydrogen peroxide, has recently been shown to act also on monoethyl hydrogen peroxide, though to a much smaller degree.[27] But even so, this enzyme will not decompose *all* simple inorganic and organic peroxides. Neither diethyl peroxide nor persulfuric acid is attacked, while peracetic acid and monomethyl hydroxyperoxide, like monoethyl hydrogen peroxide, form spectroscopically defined complexes with concentrated catalase solutions which break down spontaneously into free enzyme and split products (Stern, unpublished observations). The fact that an enzyme is named after a certain substrate no longer implies that its action is restricted to it. Tyrosinase, for instance, represents a monophenolase. It catalyzes the formation of *o*-diphenols from hydrated monophenols by dehydrogenation. Consequently, tyrosinase will attack many phenols where a second hydroxyl group may be introduced into the molecule in ortho position to the first one. The *o*-diphenols thus formed are not stable and are transformed into higher oxidation products via the corresponding *o*-quinones. Conversely, the observation that a given enzyme preparation is capable of attacking several substrates is not necessarily an indication that it contains a mixture of different enzymes, although the burden of proof for enzymatic homogeneity still rests with the investigator. If by fractionation and purification no change is accomplished in the activity ratio toward the various substrates, it may be considered evidence in favor of the multiple specificity of the enzyme under study. The mere fact that the enzyme preparation employed was in the form of a crystalline protein material, is insufficient evidence. Thus, urease crystals not further purified by recrystallization are often contaminated with arginase; and crude pepsin crystals have been shown to contain a separate, gelatin-liquefying catalyst.

The fact that the structure of the substrates decomposed by an enzyme preparation is very different, is often but not invariably an indication that we are dealing with a mixture of enzymes. The principle contained in milk which is capable of attacking xanthine and hypoxanthine (xanthine oxidase) as well as aldehydes (Schardinger enzyme) has withstood all attempts at separation so that the majority of investigators attribute both catalytic functions to one and the same enzyme. On the other hand, the claim[28] that a single active protein, namely, the so-called reducing enzyme of fermentation of Warburg, catalyzes the reduction of acetaldehyde to alcohol and also of dihydroxyacetone phosphate to glycerophosphate by dihydrocozymase appears to be insufficiently substantiated.

---

[27] K. G. Stern, *J. Biol. Chem.*, **114**, 483 (1936); *Enzymologia*, **4**, 145 (1937).

The term *relative specificity* denotes that, while an enzyme attacks a certain substrate or type of linkage preferentially, it will also act on other substrates and related linkages to a lesser degree. The majority of known enzymes seem to be in this category. The enzymes hydrolyzing (and resynthesizing) esters of fatty acids are commonly subdivided into *esterases* and *lipases* to indicate that the one group will act on the esters of the lower and the other on the esters, and especially the glycerides, of the higher aliphatic acids. The dividing line between these two groups, however, is not always very sharply defined, and it is more accurate to state that the esterases show a preference for the lower members and the lipases for the higher members of the fatty acid group, with a gradual tapering off of the activity toward the longer or shorter carbon chains, respectively. Many similar instances of relative specificity could be quoted. The specificity of enzymes, being their outstanding property, serves as the basis for their classification. Since many enzymes have not as yet been isolated as chemical entities or even been purified to a significant extent, the chief evidence for their very existence rests on observations of the cleavage or oxidation of certain test substrates by living cells or cell-free preparations. It is evident, therefore, that the demarcation between individual enzymes belonging to one general group is only as clearly defined as are the results obtained with the aid of a series of different substrates. Any advances made in specificity studies are bound to affect the system developed for the classification of that group. This situation is well illustrated by recent developments in the field of proteolytic enzymes. Based on the researches of Emil Fischer, Abderhalden, Waldschmidt-Leitz, Grassmann, and others, the following classification of proteases had been developed and widely accepted until very recently. Two main groups of proteolytic enzymes were recognized, namely, the *proteinases* and *peptidases*. The former were considered to be restricted in their action to high-molecular proteins, and they were further subdivided into the categories of pepsinases, tryptases, and papainases (or the "third group"), depending as to whether their *p*H optimum indicated the attack of protein cations, anions, or zwitter ions, respectively. The peptidases, on the other hand, were subdivided into dipeptidases and polypeptidases. Among the latter, finally, one used to distinguish between amino- and carboxypolypeptidases, depending on whether a free terminal NH_2 or COOH group was a prerequisite for the action of the respective enzyme. This entire system, where the chief emphasis was placed on the molecular weight or chain length of the substrates, has now been questioned and thrown open to drastic revision by Max Bergmann and his associates on the basis of their experiments with synthetic substrates. These were made possible by the increase in range of available synthetic peptides thanks to the ingenious carbobenzoxy method of Bergmann and Zervas. (See page 138.) There exists now some doubt as to the existence of a separate dipeptidase, since some well-defined polypeptidase preparations have been found to attack dipeptides also. Thus, crystalline carboxypolypeptidase will also hydrolyze the dipeptide *l*-tyrosyl-*l*-tyrosine (Hofmann and Bergmann[29]). According to Bergmann,[30] the specificity of the peptidases is adapted to the nature of the side groups, *i. e.*, the atomic groups which are different for different α-amino acids, rather than to the number of amino acid residues contained in a peptide. Whereas Bergmann states that the information available at present neither proves nor disproves the existence of a special dipeptidase or a class of dipeptidases, other workers, like Johnson,[31] believe in the reality of a number of dipeptidases.

---

[28] E. Negelein and H. Broemel, *Biochem. Z.*, **303**, 231 (1939).

A similar situation seems to exist with respect to the so-called proteinases. It has been reported by Bergmann and Fruton,[32] and others[30] that trypsin, chymotrypsin, pepsin, and cathepsins are able to split not only the high-molecular proteins for which they were formerly thought to be specific but also certain low-molecular, synthetic substrates containing the requisite side groups. Since the rate of breakdown of these synthetic peptides is of the same order as that of genuine proteins, it would seem that the old criterion of molecular size must now be replaced by that of the specific configuration of the substrates involved. It appears that the different proteolytic enzymes, while invariably attacking the substrate at a peptide bond (—CO—NH—), require for their action the location of other groups or bonds at a certain distance from the linkage which they resolve by hydrolysis. Depending on whether these auxiliary groups or bonds are situated within the peptide chain or whether they represent terminal groups, Bergmann differentiates between *endopeptidases* and *exopeptidases*. Thus, in order to be subject to the action of aminopeptidases, a peptide must possess a free amino group in close proximity to the carbonyl of the peptide bond which it is to hydrolyze. Substrates for carboxypeptidases must contain a free carboxyl group in addition to the imido group of the peptide bond on which they are to act. Proteinases, on the other hand, require another peptide bond near the one which they are to split or, when acting in the direction of synthesis, which they are to form. The fundamental difference, then, between amino- and carboxypeptidases on the one side and the proteinases on the other would seem to be, according to this view, that the former act on *terminal* peptide bonds, only, while the latter can attack both terminal and *centrally located* peptide linkages. Bergmann proposes the terms *exopeptidases* and *endopeptidases* for these two main groups of proteolytic enzymes. He favors the abandonment of the old terms peptidase and proteinase since the criterion of which they were based (molecular weight of the substrate) is no longer valid. While these new terms appear to express adequately the specificity requirements of the two classes of proteolytic enzymes, there is a certain risk of confusion with the differentiation of proteolytic enzymes into intra-

---

[29] K. Hofmann and M. Bergmann, *J. Biol. Chem.*, **134,** 225 (1940).

[30] M. Bergmann in *Advances in Enzymology,* Vol. II. Interscience, New York, 1942, p. 49.

[31] M. J. Johnson and J. Berger in *Advances in Enzymology,* Vol. II. Interscience, New York, 1942, p. 69.

[32] M. Bergmann and J. S. Fruton in *Advances in Enzymology,* Vol. I. Interscience, New York, 1941, p. 63.

and extracellular enzymes for which the designations of endo- and exoproteases have sometimes been used in the literature. Furthermore, Willstätter has used the term endo-enzyme for intracellular enzymes which are released upon breaking open the cell membrane, as contrasted to desmo-enzymes which are linked up to the cytoplasm and may only be "pried off" by chemical agents such as acid or alkali or enzymatic digestion.[33]

For a detailed discussion of the specificity problem of proteolytic enzymes the reader is referred to two recent review articles by Bergmann and Fruton,[30, 32] where many data in tabulated form may be found. The specificity theory here outlined is in agreement with Euler's "double-affinity" hypothesis,[34] according to which the enzyme must unite with two points rather than one spot in the substrate molecule prior to successful catalysis of its fission.

III. ENZYMES AS CHEMICAL COMPOUNDS

Long before any enzyme had been isolated in pure form, enzymes were believed to be proteins or protein-like substances by the majority of investigators. Liebig had already classified enzymes (or ferments) as albuminoid materials and his view found almost general acceptance. The evidence gathered in support of this hypothesis, although admittedly circumstantial, consisted in the behavior of enzymes (or rather their activity) toward temperature, hydrogen-ion concentration, acids, alkalies, and protein-precipitating reagents such as organic solvents, heavy metal salts, etc. Only proteins share with the enzymes their pronounced thermolability, the high critical increment of heat inactivation, and the profound influence of *p*H on their activity or solubility. Their colloidal character was proved by their inability to pass semipermeable membranes and their slow rate of diffusion; and their nature as amphoteric electrolytes was indicated by the behavior of enzymes in electrical fields as a function of the *p*H and the demonstration by this method that they possess an isoelectric point. On the other hand, Willstätter, as recently as 1926, expressed the belief that enzymes are neither proteins nor carbohydrates nor members of any known class of organic substances.[35] He was prompted to this postulate by his extensive experience gathered in attempts to purify and isolate enzymes and determine their structure. While he and his associates were able to purify many enzymes by elaborate methods, involving chiefly adsorption on inorganic gels and elution from them, they found in general that the absolute activity diminished as the purity of the preparations, *i. e.*, the activity/dry weight ratio, increased. Finally, the activity was frequently lost altogether, an observation which was explained by assuming that pure enzymes are extremely unstable and that they require the presence of certain auxiliary substances for their existence. It was later shown that adsorption and elution methods, while often suitable for the separation of different enzymatic activities from each other, are in general not the best technique for preparative purposes, where high yields are essential, especially if many successive steps in the purification of an enzyme are required. The first instance where an enzyme was obtained as an entity was the isolation of *urease* by Sumner[36] in 1926. He obtained the enzyme in form of a crystalline protein by the simple expedient of extracting Jack bean meal with 30% aqueous acetone and subsequent cooling of the filtrate. The protein could be further purified by recrystallization. The procedure was so extremely simple that the discovery was received with a good deal of skepticism, especially in Europe; that is, nobody doubted that a crystalline material endowed with urease activity could be prepared in this way, but it took some time before it was generally conceded that the crystalline protein is identical with the enzyme itself rather than an impurity which contains traces of active enzyme adsorbed on it. It is interesting to note that the general reaction toward Stanley's announcement, in 1935, that he had isolated tobacco mosaic virus in the form of a crystalline protein, was much the same as that in the instance of urease. The psychological difficulty was, of course, in both cases that an elusive biological agent which, through the long years of efforts to elucidate its nature, had acquired a certain halo, was unexpectedly exposed as a material of familiar type which occurs in suitable sources in quantities which may be considerable. Thus, Stanley and his associates have prepared the virus protein in amounts probably exceeding 1 kg., and the same is true for a number of crystalline enzymes. Sumner's discovery and the conclusions which he drew from it became more widely accepted with the isolation of pepsin and trypsin in the form of crystalline proteins by Northrop and Kunitz[37] in 1929 and 1931, respectively. The hope, however, that with the isolation of enzymes in pure form the veil of mystery surrounding the problem of enzyme action would be lifted did not materialize. It may be stated that the crystallization of enzymes in the form of active proteins has neither enhanced materially our knowledge of enzyme action nor of enzyme structure. It has merely relegated the latter to the general problem of *protein* structure, which still awaits its complete solution. (See page 163.)

---

[33] C. Oppenheimer, Supplement to *Die Fermente und ihre Wirkungen.* Junk, Amsterdam, 1936–1939, pp. 208 and 401.

[34] H. v. Euler and K. Josephson, *Z. physiol. Chem.*, **157**, 122 (1926).

[35] R. Willstätter, *Ber.*, **59**, 1 (1926).

1. Enzymes as "Simple" Proteins

The following enzymes have been isolated in recent years in the form of crystalline, "straight" proteins: urease (Sumner, 1926), pepsin (Northrop, 1930), amylase (Caldwell *et al.*, 1931), trypsin (Northrop and Kunitz, 1932), chymotrypsin (Kunitz and Northrop, 1935), carboxypeptidase (Anson, 1935), ficin (Walti, 1937), papain (Balls *et al.*, 1937), lysozyme (Abraham and Robinson, 1937), chymopapain (Balls and Lineweaver, 1939), and starch phosphorylase (Cori, 1942). For a detailed description of the proteolytic members of the above series of enzymes, reference is made to Northrop's monograph on crystalline enzymes.[37] In none of these instances has conclusive evidence for the existence of a prosthetic group been found, *i. e.*, of a characteristic, nonprotein group attached to the protein molecule. Whenever a catalytically active protein has been found to contain such a prosthetic group, it has been possible to show that it represents the active group of the catalyst; in other words, that it combines or reacts with the substrate in a manner which explains the specific type of activity of the enzyme (see page 219). This does not mean that the protein, to which the prosthetic group is more or less firmly attached, is merely an unspecific colloidal carrier for the active group. It is generally recognized that the protein is an indispensable component and that it influences profoundly the level and type of catalytic activity of the enzyme simplex. In those cases where no distinct prosthetic group can be detected, we have to conclude that the entire configuration of the amino acid components and of the peptide chains which they form within the protein molecule, is involved in the enzymatic function, although certain regions of the macromolecule are probably of greater relative importance than others. Attempts to isolate the latter regions, which Frankenburger has aptly called the "active cross section" of the enzyme, lead of necessity to the breakdown of the entire native protein structure and, consequently, to inactive fragments. The situation may be compared to the problem of removing a mural without damaging the wall on which it is painted. In such instances, where catalytic function is seemingly tied up with protein structure in an indissoluble manner, another experimental approach to the problem of functional groupings has been more successful. It consists in the systematic blocking of certain terminal groups, *e. g.*, free amino, carboxyl, or hydroxyl groups by specific reagents and in studying the enzymatic activity of the resulting protein derivative. Herriott, in Northrop's laboratory, has shown in this manner that the free amino groups of pepsin are not necessary for its enzymatic activity whereas the phenolic hydroxyl groups on the aromatic amino acid residues in the molecule, on the other hand, are indispensable for its biological activity. The special importance of the tyrosine component of pepsin is further emphasized by the fact that introduction of iodine into the benzenoid portion of this amino acid, while it is contained in the pepsin molecule, and the resulting production of the diiodotyrosine configuration cause a decline in proteolytic activity (Herriott).[38]

---

[36] J. B. Sumner, *J. Biol. Chem.*, 69, 435 (1926).

[37] J. H. Northrop, *Crystalline Enzymes.* Columbia Univ. Press, New York, 1939.

It should be mentioned that in addition to the proteolytic enzymes mentioned above, several inactive precursors have also been isolated in the form of crystalline proteins. They are chymotrypsinogen (Kunitz and Northrop, 1935), trypsinogen (Kunitz and Northrop, 1936), and pepsinogen (Herriott, 1938). The exact structural differences between these precursors and the active enzymes, which may arise from them under suitable conditions, are still obscure.

In partly activated pancreatic extracts more or less active trypsin occurs in the form of an enzymatically inactive complex with a low-molecular inhibitor of polypeptide nature. The inhibitor-trypsin compound, as well as the free inhibitor, has been isolated in the form of characteristic crystals (Kunitz and Northrop, 1936). The inactive complex is not identical with the inactive precursor, trypsinogen.

The role played by the various secreted proteolytic enzymes in the digestion of foodstuffs is discussed elsewhere (see page 327 *et seq.*).

---

[38] J. H. Northrop, *Crystalline Enzymes*. Columbia Univ. Press, New York, 1939.

It is possible that some of the hydrolytic enzymes which in the past have been regarded as "straight" proteins may actually represent in their fully active form complexes between a protein and a nonprotein component. Thus, Linderstrøm-Lang has demonstrated the activating effect of zinc ions on dipeptidase, and Johnson, as well as Maschmann, has been able to increase the activity of a similar or of the same intestinal enzyme toward *l*-leucylglycine by the addition of manganese or magnesium salts. The recent observation by Smith and Bergmann[39] that the active enzyme may be inactivated by dialysis and that the activity may be restored by adding manganese or magnesium salts, suggests that the active enzyme is a dissociable metal-protein complex. The well-known fact that certain proteolytic enzymes of the papainase type may be activated by treatment with hydrogen cyanide and others with hydrogen sulfide is likewise interpreted by Bergmann[39] as evidence that the active enzymes are composed of an inactive protein bearer (apoenzyme in Euler's terminology) and a low-molecular activator. Arginase, according to Hellerman, Edlbacher, and other authors, contains manganese as an essential constituent. The role of heavy metals in hydrolytic enzymes, in contrast to their function in oxidative enzymes, is not yet understood.

2. Enzymes as Conjugated Proteins

All oxidative enzymes, the chemical nature of which has thus far been ascertained either by direct or by indirect methods, have been found to contain special, prosthetic groups in addition to their protein components. They may be classified, according to the structure of their prosthetic groups, as *metalloproteins*, *flavoproteins* (or alloxazine proteins), *pyridinoproteins*, and *thiaminoproteins*. The first class may again be subdivided into iron, copper, and zinc proteins. The iron protein enzymes thus far studied in detail contain, in addition to the heavy metal, also porphyrin groups. Since the iron is linked to the nitrogen atoms of the tetrapyrrol skeleton of porphyrin nuclei, these enzymes have also been designated as *hemin proteins* and they are, therefore, built in a manner analogous to the blood pigment, hemoglobin. There is a further subdivision within this group, depending on whether the hemin is a so-called red hemin, in this case protohemin, or whether it is a mixed-colored hemin (pheohemin in Warburg's terminology). The group of protohemin enzymes includes the classical peroxidases, the recently discovered cytochrome peroxidase (Hogness *et al.*), and the catalases. The respiratory ferment of Warburg which is held to be identical with cytochrome oxidase, and the Pasteur enzyme (Stern and Melnick) which represents the catalyst responsible for the inhibition of fermentation by oxygen are pheohemin enzymes. A recent discussion of some properties of these catalysts and of their mode of action will be found in a symposium volume on respiratory enzymes (Stern[40]). The group of Keilin's intracellular cytochrome pigments (identical with MacMunn's histohematins) belongs also in the class of hemin proteins. But it is doubtful whether the cytochromes should be considered as enzymes or as nonenzymatic intermediate catalysts. They are the connecting links between the respiratory enzyme or cytochrome oxidase, on the one hand, and flavoprotein and pyridinoprotein enzymes, on the other hand. We distinguish between three types of cytochromes, namely, a, b, and c. These again occur in nature in several modifications, such as the a_1, a_2, a_3, b, b_1, b_2, c and c_1 components which may be discerned by their characteristic absorption spectra. The b- and c-cytochromes contain red hemin groups, while the a-components contain mixed-colored and perhaps even green or chlorophyll hemin residues. It is possible but not proved that cytochrome a_3 is identical with cytochrome oxidase (Keilin). The present status of the cytochrome problem has recently been reviewed by Stotz.[41]

---

[39] M. Bergmann in *Advances in Enzymology*, Vol. II. Interscience, New York, 1942, p. 49.

[40] K. G. Stern, *Symposium on Respiratory Enzymes*. Univ. of Wisconsin Press, Madison, 1942, p. 74; see also C. Oppenheimer and K. G. Stern, *Biological Oxidation*. Junk, Amsterdam, 1939.

Several phenol- and polyphenoloxidases have been identified as *copper proteins.* They include a polyphenol oxidase from potatoes (Kubowitz), from cultivated mushrooms (Keilin and Mann), laccase (Keilin and Mann), and a monophenol oxidase from wild mushrooms (Dalton and Nelson). These enzymes contain no porphyrin residues and the copper is bound to the protein in a manner analogous to its linkage in hemocyanin, the blood pigment of certain invertebrates. Carbonic anhydrase, the enzyme catalyzing the equilibrium $CO_2 + H_2O \rightleftarrows H_2CO_3$, is a *zinc protein* (Keilin and Mann). It may play a role in the hydrochloric acid production in the stomach (see page 334).

Ascorbic acid oxidase, an enzyme widely distributed in plant tissues, is probably a copper protein. The same holds with regard to tyrosinase, the enzyme in potatoes and other biological materials which initiates the oxidation of tyrosine and related compounds to melanin.

The important class of *flavoproteins* may be subdivided into enzymes which have riboflavinphosphate and those which have flavinadenine dinucleotide in their prosthetic groups. The structure of these groups will be given in the section devoted to coenzymes (see page 219). Members of this class are Warburg's so-called "old" yellow enzyme, which catalyzes the oxidation of hexosemonophosphate to phosphogluconic acid in cooperation with coenzyme II and Warburg's "zwischenferment" (see page 223), Krebs' *d*-amino acid oxidase from animal tissues, xanthine oxidase (Ball), fumaric hydrogenase (F. G. Fischer), cytochrome-c reductase (Haas and Hogness), and other oxidative enzymes. Most of these enzymes may be reversibly dissociated into their prosthetic alloxazine group and their protein component. In some instances, the colored group isolated from one flavoprotein could be coupled with the protein component of another flavoprotein, yielding an enzyme endowed with new properties. The number of known flavoproteins has rapidly increased during the past 10 years and is still growing (see Hogness[42]).

[41] E. Stotz, *Symposium on Respiratory Enzymes.* Univ. of Wisconsin Press, Madison, 1942, p. 149.

[42] T. R. Hogness, *Symposium on Respiratory Enzymes.* Univ. of Wisconsin Press, Madison, 1942, p. 134.

Even more numerous than the preceding classes of conjugated enzyme proteins are the members of the group of *pyridinoproteins*. As prosthetic group, they contain either coenzyme I (or cozymase) or coenzyme II. There are present in animal and plant cells and in microorganisms proteins which are capable of forming active enzyme systems of high and pronounced specificity with one or the other of these two pyridine coenzymes. The main characteristic of this group of oxidative enzymes, or rather dehydrogenases, is that they constitute reversibly dissociable systems and that a small number of protein molecules are thereby enabled to catalyze the reaction of many substrate molecules with fresh coenzyme molecules in a given time interval. This explains why in natural sources such as yeast the coenzymes occur in a large excess with regard to the protein components of these enzyme systems.

Among the pyridinoprotein enzymes are the lactic and malic dehydrogenases in animal tissues, the enzyme catalyzing the oxidation of alcohol to aldehyde and the reverse reaction, variously designated as alcohol dehydrogenase, acetaldehyde reductase, and Warburg's reducing fermentation enzyme, *l* (+)-glutamic dehydrogenase, aldehyde mutase; furthermore the enzyme catalyzing the oxidation of phosphoglyceraldehyde to phosphoglyceric acid with the intermediate formation of diphosphoglyceric acid (triosephosphoric enzyme or Warburg's oxidizing fermentation enzyme), the glycerophosphoric enzyme in muscle tissue, hexosemonophosphoric dehydrogenase (Warburg's "zwischenferment" plus coenzyme II), phosphogluconic dehydrogenase, isocitric dehydrogenase, and others (see Schlenk[43]).

The fact that many of these pyridinoproteins operate with the same coenzyme (either cozymase or coenzyme II) and differ only with regard to their protein components, and the further fact that in some cases the forward (oxidizing) reaction, in others the back (reducing) reaction, is favored by the position of the equilibrium point, enables the pyridinoproteins to interact in a manner which has been described as a *coenzyme-linked reaction*. Such reactions take place if the coenzyme is reduced by reacting with one substrate on the surface of one active protein and is subsequently oxidized with the aid of another substrate and a second protein. Examples are the oxidation of lactate to pyruvate at the expense of oxaloacetate which is thereby reduced to malate, or the oxidation of β-hydroxybutyrate to acetoacetate with the concomitant reduction of aldehyde to alcohol. The equilibrium position for the reaction of pyridinoproteins with their coenzymes and substrates can also be expressed in terms of oxidation-reduction potentials. It is obvious that a coupled or coenzyme-linked reaction of the kind mentioned above can only occur at a measurable rate if one of the two reaction partners has a normal potential which is markedly more positive than that of the other half. A discussion of much recent work on pyridinoproteins will be found in Green's useful and compact monograph on mechanisms of biological oxidations.[44] For an exposition of the subject of oxidation-reduction potentials with particular reference to biological systems the reader is referred to the monograph by Michaelis[45] and two brief surveys by the writer.[46, 47]

---

[43] F. Schlenk, *Symposium on Respiratory Enzymes.* Univ. of Wisconsin Press, Madison, 1942, p. 104.

[44] D. E. Green, *Mechanisms of Biological Oxidations.* Macmillan, New York, 1940.

The last group of enzymes, representing conjugated proteins, which have to be mentioned at this time are the *thiaminoproteins*. Their prosthetic group consists of thiaminepyrophosphate. Thus far, only two representatives of this group have been described, namely, yeast carboxylase which in purified form constitutes a thiamino-magnesium-protein (Green) and pyruvic dehydrogenase, an enzyme present in certain bacteria and animal tissues. Carboxylase brings about the anaerobic decomposition of pyruvic acid into carbon dioxide and acetaldehyde, involving the secondary formation of some acetoin, while pyruvic dehydrogenase, operating in conjunction with flavoproteins and other auxiliary catalysts, promotes the oxidative decarboxylation of the same substrate to acetic acid and carbon dioxide. The detailed mechanism of carboxylase action is still obscure.[48] In the case of pyruvic dehydrogenase, Lipmann[49] has been able to make out a strong case for the intermediate formation of acetylphosphate during the reaction.

3. Structure and Function of Coenzymes

Coenzymes may be defined as low-molecular organic substances which are capable of supplementing specific proteins to form active enzyme systems. If this definition be accepted then the term coenzyme becomes synonymous with "prosthetic group," at least as far as enzymatically active conjugated proteins are concerned. The hemin residue of hemoglobin cannot be called a coenzyme because hemoglobin is a respiratory transport substance rather than an enzyme. The same is true for the nucleic acid residues of nucleoproteins. In adopting this terminology, the previously erected artificial barrier, separating the readily dissociable from the difficultly dissociable conjugated protein enzymes, would fall in the interest of consistency and uniformity of nomenclature.

The chemical formulas of some coenzymes are given in Fig. 15. The formulas of some other coenzymes will be found on pages 170 and 171.

The prosthetic group of catalase and very probably also of peroxidase is protoferriheme IX, *i. e.*, the ferric iron complex salt of 1,3,5,8-tetramethyl-2,4-divinylporphine-6,7-dipropionic acid. In the active enzymes, the metal is in the trivalent state, and though it may be reduced to the ferrous form under certain conditions there is as yet lacking adequate experimental proof that the iron oscillates from the ferric to the ferrous and back to the ferric state during the catalyses. The hemin residues are rather firmly attached to the protein bearer in these enzymes. Their detachment by acids etc., leads to inactivation which thus far has not been reversed in the instance of catalase. The first step in the catalysis promoted by these enzymes consists in a combination with the substrate to form labile complexes which in some cases may be observed by direct spectroscopy. (Stern, Keilin).

---

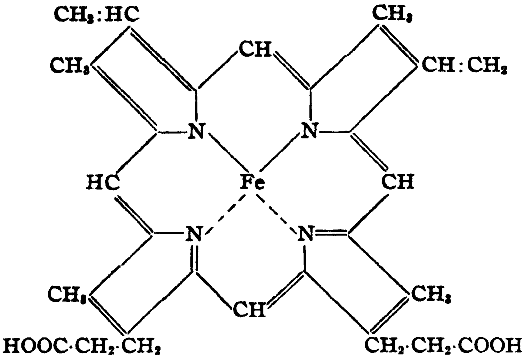
[45] L. Michaelis, *Oxidations-Reductions Potentiale*. Springer, Berlin, 1933.

[46] K. G. Stern, *Tab. Biol.*, **10**, 1, 353 (1934–1935).

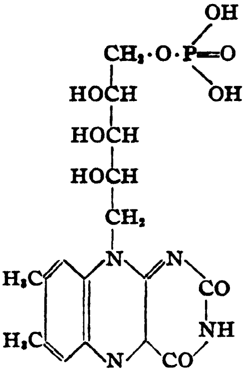
[47] K. G. Stern, *Handbook of Medical Physics*. In press.

[48] K. G. Stern and J. L. Melnick, *J. Biol. Chem.*, **131**, 597, 615 (1939); **135**, 365 (1940); *Enzymologia*, **8**, 129 (1940).

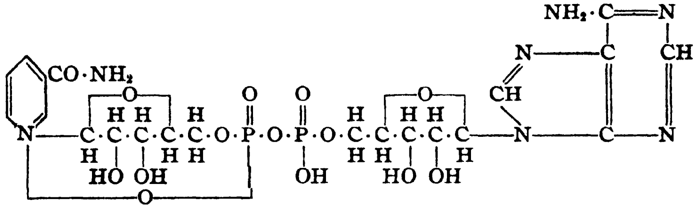
[49] F. Lipmann, *J. Biol. Chem.*, **134**, 463 (1940).



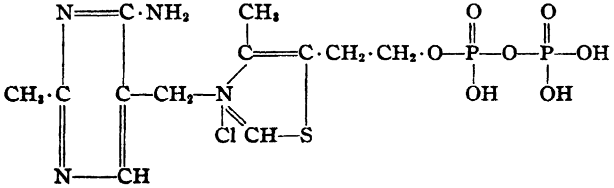
Protoferriheme IX



Alloxazine nucleotide (riboflavin phosphoric acid)



Diphosphopyridine nucleotide (cozymase)



Pyrimidinethiazolepyrophosphoric acid (cocarboxylase)

Fig. 15.—Structure of some coenzymes.

The prosthetic groups of the oxygen-transferring enzyme of Warburg as well as of the Pasteur enzyme are pheohemins, *i. e.*, identical with or isomers of spirographis hemin (iron complex salt of 1,3,5,8-tetramethyl-2-formyl-4-vinylporphine-6,7-propionic acid). In the course of their action, these hemin groups shuttle back and forth between the ferrous and ferric form. The primary step upon reaction with molecular oxygen consists probably in the formation of an oxygen adduct comparable to oxyhemoglobin. This is followed by intramolecular oxidation to the ferric state which is analogous to methemoglobin. The ferric iron of the enzymes is the true oxidizing agent for the next catalyst in the chain which, in the instance of the respiratory enzyme, is very probably cytochrome c. The latter, in turn, is oxidized to the ferric form and now oxidizes the following catalyst, such as cytochrome-c reductase. Both types of enzymes are present in the living cell in only minute concentration and it is doubtful whether they may be obtained in solution in fully active and unchanged form. They seem to be anchored to macromolecular particles or to structural elements of the cell.

The prosthetic group of cytochrome c is a modified protohemin which is linked to the protein component by a relatively stable thioether bridge. The configuration of the other cytochrome components has not yet been established with certainty.

The flavoproteins contain either riboflavinphosphate (6,7-dimethyl-9-*d*-ribitylisoalloxazine-5′-phosphoric acid) or flavinadenine dinucleotide which contains riboflavinphosphate and adenylic acid in a nucleotide-like configuration. Their linkage with various specific proteins is of varying stability and may be dissolved in a reversible manner. In the course of their action, these coenzymes are reversibly reduced to leucoforms via semiquinoid monohydro radicals. Each flavoprotein has probably a characteristic oxidation-reduction potential which has, however, thus far only been measured experimentally for the instance of the original yellow enzyme of Warburg which contains riboflavinphosphate. Flavoproteins may react directly with certain substrates whereas they require the cooperation of other enzymes such as pyridinoproteins and/or hemin proteins in other instances.

The two coenzymes encountered in pyridinoproteins, which are designated as coenzyme I (cozymase) and coenzyme II, respectively, or as codehydrogenase I and II in Euler's terminology, contain nicotinic acid amide, pentose, adenine, and phosphoric acid residues. Cozymase (coenzyme I) is a diphosphopyridine nucleotide, whereas coenzyme II is a triphosphopyridine nucleotide. The pentose component has recently been identified as ribose. In the course of their catalytic action, the pyridine coenzymes are reversibly reduced to the corresponding dihydro forms and reoxidized, possibly via a radical-like monohydro intermediate. The reduction and oxidation is localized in the pyridine portion of the molecule, the double bond between the quaternary nitrogen atom of the pyridine ring and an adjacent carbon atom being reversibly hydrogenated. Both coenzymes probably contain only two phosphoric acid groups lined up in series, *i. e.*, in pyrophosphate configuration. The third phosphoric acid residue present in coenzyme II is probably attached to one of the ribose components in the form of a side chain. The two pyridine coenzymes may be converted into each other and may substitute for one another in certain dehydrogenase systems. Other dehydrogenases exhibit an absolute specificity with regard to the coenzyme. The linkage between these coenzymes and the active proteins is rather loose and is often dissociated upon mere dilution. There is a marked difference in affinity to the protein between the oxidized and the reduced forms of the coenzymes.

The chemical transformations which cocarboxylase (pyrimidinethiazolepyrophosphate or thiaminepyrophosphate) undergoes in the course of the action of car-

TABLE 26

ENZYMES AS CONJUGATED PROTEINS

Prosthetic group or coenzymes	Name of complete enzyme system	Chief substrates attacked	End products formed	Remarks
I. Metalloproteins (*a*) Hemin proteins				
Protoferriheme IX (+ side chains)	Catalase	Hydrogen peroxide	Water, oxygen	
	Peroxidase	H_2O_2 + H-acceptors	Water, oxidized pigments	
	Cytochrome c	Reduced flavoproteins	....	Intermediate catalyst
	Cytochrome c peroxidase	H_2O_2 + ferrocytochrome c	Water + ferricytochrome c	
Pheohemin	Respiratory ferment (cytochrome oxidase)	O_2, ferrocytochrome c	Water, ferricytochrome	
	Pasteur enzyme	O_2, component of fermentation system	....	**Inhibitor of fermentation**
(*b*) Copper proteins				
Copper in unknown linkage	Polyphenol oxidase	O_2 + *o*-diphenols	*o*-Quinones	
	Laccase	O_2 + *p*-phenylenediamine	Pigments	**Natural substrate laccol**
	Tyrosinase	O_2 + tyrosine, dopa	Melanins	
	Ascorbic oxidase	O_2 + ascorbic acid	Dehydroascorbic acid	
(*c*) Zinc proteins				
Zinc in unknown linkage	Carbonic anhydrase	$CO_2 + H_2O \rightleftharpoons$	Carbonic acid	
	Insulin	Intermediate in carbohydrate metabolism	?	**Enzymatic nature doubtful**

Table 26 (*Continued*)

Enzymes as Conjugated Proteins

Prosthetic group or coenzymes	Name of complete enzyme system	Chief substrates attacked	End products formed	Remarks
II. Flavoproteins				
Riboflavinphosphate	"Old" yellow enzyme	Hexosemonophosphate *via* red. coenzyme II	Phosphohexonic acid	(Artifact?)
	Cytochrome c reductase	Cytochrome c + red. coenzyme II	Red. cytochrome c + coenzyme II	
Flavinadeninedinucleotide	"New" yellow enzyme	Methylene blue + red. coenzyme I	Leucomethylene blue + coenzyme I	
(+ 2nd prosthetic group?)	*d*-Amino acid oxidase	O_2, *d*-amino acids	Keto acids	
	Xanthine oxidase	Xanthine, aldehydes + oxygen	Uric acid, acetic acid	
	Fumaric hydrogenase	Leucodyes + fumaric acid	Succinic acid	
III. Pyridinoproteins				
Coenzyme I (cozymase)	Alcohol dehydrogenase (acetaldehyde reductase)	Acetaldehyde ⇄	Alcohol	
	Lactic dehydrogenase	Lactic acid ⇄	Pyruvic acid	
	Hydroxybutyric acid dehydrogenase	β-hydroxybutyric acid	Acetoacetic acid	In animal tissues
Coenzyme II	Glutamic dehydrogenase	1 (+)-Glutamic acid	α-Ketoglutaric acid + NH_3	
	Hexosemonophosphoric dehydrogenase	Glucose-6-phosphate	6-Phosphogluconic acid	
	Isocitric dehydrogenase	Isocitric acid	α-Ketoglutaric acid	
IV. Thiaminoproteins				
Cocarboxylase (diphosphothiamine)	Carboxylase	α-Keto acids	Aldehydes + CO_2	Yeast
	Pyruvic dehydrogenase	α-Keto acids + O_2	Fatty acid + CO_2	Bacteria, animals

boxylase or pyruvic dehydrogenase are still unknown. Although the coenzyme may be reduced in a rather sluggish manner *in vitro* by suitable reagents, the catalytic activity of the reduced coenzyme is very doubtful. The chemical behavior of the amino group makes it unlikely that the mechanism proposed by Langenbeck for certain carboxylase models and hence called the "Langenbeck cycle" is operative during the enzymatic action of these enzymes.[50] Details concerning the chemistry of these coenzymes may be found in a recent monograph on biological oxidation.[51] The relationship between such prosthetic groups and protein carriers is discussed in that monograph as well as in three fairly recent review articles.[52–54]

A survey of the various types of enzymes representing conjugated proteins and of the coenzymes which they contain will be found in Table 26.

4. Relationships between Enzymes, Vitamins, and Hormones

For some time, the enzymes, hormones, vitamins, essential trace elements, viruses, etc., have been grouped together under the common heading of *biocatalysts*. Even without a detailed knowledge of the mechanism of action of some of these agents, such a general classification appears justified in view of the fact that they exert a powerful biological effect of pronounced specificity in amounts which often represent mere traces and which never approach the concentration levels of substances which are recognized building blocks of living cells or sources of caloric energy, *e. g.*, proteins, fats, or carbohydrates. It has also been common usage to designate the effective concentrations of these agents as catalytic amounts, thereby implying that their mode of action, in the last analysis, is concerned with catalytic processes. Only in the last few years, however, has experimental evidence in support of this working hypothesis become available. The most spectacular successes in this regard have been scored in the field of members of the vitamin B complex. It has been possible to show that several of these vitamins, which are discussed in detail (see page 229), are constituents of important enzyme systems. Thus, vitamin B_2 (riboflavin) forms part of the prosthetic group of the alloxazine protein enzymes, while vitamin B_1 (thiamine) is a component of cocarboxylase and therefore also of carboxylase and pyruvic dehydrogenase. Nicotinic acid or the corresponding amide (antipellagra vitamin) is required for the synthesis of the pyridine coenzymes, cozymase and coenzyme II. It is of interest to note that these vitamins are usually introduced into the animal organism in the free state while the corresponding coenzymes contain phosphoric acid radicals. The coupling with phosphate groups, which is a necessary prerequisite to their interaction with the specific enzymatic proteins, is brought about by active phosphorylating enzymes present in the intestinal wall and also in the tissues themselves where the coenzymes are finally operative. The chemical relationships between these vitamins and the corresponding coenzymes are evident from an inspection of Fig. 15. It is seen that nicotinic acid represents only a small though very important portion of the cozymase molecule.

---

[50] K. G. Stern and J. L. Melnick, *J. Biol. Chem.*, **131**, 597, 615 (1939); **135**, 365 (1940); *Enzymologia*, **8**, 129 (1940).

[51] C. Oppenheimer and K. G. Stern, *Biological Oxidation.* Junk, Amsterdam, 1939.

[52] O. Warburg, *Ergeb. Enzymforsch.*, **7**, 210 (1938).

[53] K. G. Stern, *Cold Spring Harbor Symposia*, **6**, 286 (1938).

[54] K. G. Stern, *Enzymologia*, **5**, 190 (1938).

It has been suspected, and in fact claimed for some time, that vitamin C (ascorbic acid) is also an essential constituent in enzymatic reactions in the cell. Its precise function, beyond its capacity to function as a mild reducing agent and to poise the potential of living cells by virtue of its being an oxidation-reduction system which is reversible within a fairly narrow *p*H range, is still unknown. It is possible that ascorbic acid, like glutathione and similar substances, fulfills nonenzymatic, catalytic functions in cell respiration or other cellular processes. Oppenheimer[55] has called such materials *mesocatalysts*. However, it is also possible that in the cell ascorbic acid combines with a specific protein to form an active enzyme system. Since the only derivative of vitamin C, thus far found in animal tissues, is dehydroascorbic acid, *i. e.*, the reversibly oxidized form, it is unlikely that it undergoes chemical changes comparable to those suffered by the B vitamins mentioned above prior to entering into action. The fact that some of the ascorbic acid present in plant tissues occurs in bound form is suggestive of the existence of a coenzyme-protein complex. The claim of Kraut that ascorbic acid is the prosthetic group of esterase remains unconfirmed thus far.

If one generalizes from the experience gained with regard to the above vitamin B complex components, it would appear very probable that the other members of the same complex, *e. g.*, pantothenic acid, pyridoxine (vitamin B_6), or biotin, function as coenzymes in the cell. Experimental evidence for this working hypothesis, however, is still lacking. It has been suggested that pyridoxine is concerned with the catalysis of intermediate fat metabolism and that biotin might be the coenzyme of the Wood and Werkman reaction, *i. e.*, concerned with carbon dioxide transfer (Burk). The evidence, however, is of an indirect nature, and the only positive indication is the fact that all of these vitamins occur in their natural sources partly in a bound form, presumably linked to protein. Of great interest in this connection is the inactivation of biotin by avidin, a protein occurring in small amounts in egg white. This complex is very stable, and it is possible that the vitamin is inactivated by avidin because the latter competes successfully with an active protein, present in cells, for the biotin molecule, to form with it an enzymatically inactive complex.

The relationship between enzymes and hormones is even more obscure than that between many vitamins and enzymes. The particular difficulty here is that the majority of hormones require the intact animal organism for a demonstration of their specific action. We know that insulin controls the level of glucose in the blood, but the cell physiological mechanism responsible for this effect is as difficult to derive from the study of the over-all phenomenon as would be an attempt to reconstruct the mechanics of a vending machine on the basis of the observation that the insertion of a coin into the slot causes the appearance of the merchandise in a receptable. It has, however, recently been reported by Krebs and confirmed by others that insulin, in relatively large amounts and under specialized conditions, exerts an *in vitro* effect on the oxidation of glucose by minced muscle tissue. No catalytic or enzymatic effects in well-defined test systems have as yet been demonstrated for other hormones, *e. g.*, the thyroid or hypophysis protein hormones. It is tempting, however, to speculate on the possibility that these hormones might function as the protein carriers of specific enzyme systems just as vitamins are the precursors of coenzymes.

---

[55] C. Oppenheimer and R. Kuhn, *Die Fermente und ihre Wirkungen*, Thieme, Leipzig, 1925; C. Oppenheimer, *Supplement*, Junk, Amsterdam, 1936–1939.

Euler and B. Zondek believe they have obtained indications for the enzymatic nature of prolan, one of the pituitary gonadotropic hormones, and Euler has proposed the terms *vitazymes* and ***hormozymes*** for substances like riboflavin and prolan, in keeping with the working hypothesis that such materials exert their specific pharmacodynamic function in the cell in the form of enzymes or at least analogous systems composed of an active or prosthetic group and a specific protein carrier.

5. Classification of Enzymes

Two different principles may be employed in the classification of enzymes, namely, their catalytic activity and their chemical constitution. The adoption of the latter criterion as a basis for classification is impeded by the fact that the chemical composition of many enzymes of considerable importance is still unknown. Although there have been some recent attempts to classify special types of enzymes in this manner,[56] the system in most general use at the present is based on the substrates attacked by the individual enzymes and on their type of action. The majority of the names for enzymes are derived from the name of their substrate or substrates, with the letters "-ase" added to it. These terms refer to typical substrates (or end products) rather than always to the natural ones with which the enzyme would react *in vivo*. Thus, indophenoloxidase is an enzyme which oxidizes *p*-phenylenediamine plus naphthol to indophenol with the aid of molecular oxygen. Since the enzyme responsible for this effect has been identified by Warburg and by Keilin with cytochrome oxidase (Warburg's respiratory ferment), the term indophenoloxidase is rapidly becoming obsolete. Warburg[57] has proposed to indicate, in the designation of an enzyme, both its structure and the nature of the substrate. Thus, for alcohol dehydrogenase the label diphosphopyridine-nucleotide $\text{protein}_{\text{methylene blue, acetaldehyde, alcohol}}$ is suggested. Warburg himself, however, has recognized that this terminology is somewhat cumbersome and typographically difficult, and for short he employs the name acetaldehyde reductase for this enzyme. The only justification for using such elaborate terms as alloxazine-adenine-dinucleotide $\text{protein}_{O_2,d\text{-amino acids}}$ in place of *d*-amino acid oxidase would seem to exist in instances where the chemical structure of the enzyme is actually of importance, *e. g.*, in writing equations involving the enzyme as a reactant. A system of enzyme classification, based largely on Oppenheimer's[58, 59] suggestions, is reproduced in Table 27. This system is sufficiently comprehensive as well as flexible so as to allow for revisions and additions as they become necessary through new experiments.

---

[56] D. E. Green, *Mechanisms of Biological Oxidations*. Macmillan, New York, 1940.

[57] O. Warburg, *Ergeb. Enzymforsch.*, **7**, 210 (1938).

[58] C. Oppenheimer and R. Kuhn, *Die Fermente und ihre Wirkungen*, Thieme, Leipzig, 1925; C. Oppenheimer, *Supplement*, Junk, Amsterdam, 1936–1939.

[59] C. Oppenheimer and K. G. Stern, *Biological Oxidation*. Junk, Amsterdam, 1939.

TABLE 27

CLASSIFICATION OF ENZYMES

- A. Hydrolases
  - I. Esterases
    - (a) Lipases
      - 1. Zoolipases
      - 2. Phytolipases
    - (b) Phosphatases
    - (c) Sulfatases
    - (d) Miscellaneous esterases (tannase, chlorophyllase)
  - II. Carbohydrases
    - (a) Hexosidases (oligosaccharases)
      - 1. α-Glucosidases (maltase, trehalase)
      - 2. β-Glucosidases (cellobiase, gentiobiase, phenolglucosidase)
      - 3. α-Galactosidases (melibiase)
      - 4. β-Galactosidases (lactases)
      - 5. α-Mannosidase
      - 6. β-am-Fructosidase (saccharase)
      - 7. Miscellaneous hexosidases, pentose containing glycosides, etc.
    - (b) Polyases (polysaccharases)
      - 1. Amylases
      - 2. Cellulases (including lichenase)
      - 3. Inulinase
      - 4. Other polyases (xylanase, galactanase, pectinase, gelase, chitinase, etc.)
      - 5. Nucleases
  - III. Amidases
      - 1. Acidamidases (urease, hippuricase, asparaginase)
      - 2. Purinamidases
      - 3. Arginase, histidase, tyramine oxydase
      - 4. Phosphamidases (?)
      - 5. Related enzymes (aspartase)
  - IV. Proteases
    - (a) Peptidases
      - 1. Dipeptidases
      - 2. Polypeptidases (amino- and carboxypolypeptidases)
      - 3. Protaminases
    - (b) Proteinases
      - 1. Pepsinases (stomach pepsin, proteinase of insectivore plants, etc.)
      - 2. Tryptases (pancreas trypsin, chymotrypsin, proteinases of leucocytes, insectivore plants, etc.)
      - 3. Papainases (papain, bromelin, ficin, cathepsin, etc.)
      - 4. Various bacterial proteinases of uncertain type
      - 5. Miscellaneous proteinases (chymosin, keratinase, etc.)
- B. Desmolases
  - I. Hydrokinases
    - (a) Dehydrogenases
      - 1. Anoxytropic dehydrogenases (acidodehydrogenases, hydroxyacidodehydrogenases, pyruvic-, formic-, oxalic dehydrogenase, pyridinoproteins, certain flavoproteins)
      - 2. Intermediate hydrogen carriers containing hemin (cytochromes)
      - 3. Oxytropic dehydrogenases (xanthine oxidase (Schardinger enzyme), certain other flavoproteins)
    - (b) Oxhydrases
      - 1. Glucose oxidase, ascorbic acid oxidase
      - 2. *d*-Amino acid oxidase
      - 3. Oxhydrases of amines (histaminase, tyraminase)
      - 4. Related enzymes (proline oxidase, etc.)

TABLE 27 (*Continued*)

CLASSIFICATION OF ENZYMES

- (*c*) Oxidases
    - 1. Cytochrome oxidase (respiratory ferment)
    - 2. Pasteur enzyme
    - 3. Uricase and allantoinase
    - 4. Chromo-oxidases (monophenolase, orthophenolases, paraphenolases, etc.)
    - 5 Luciferase
- II. Auxiliary Enzymes of Desmolysis
    - (*a*) Hydratases and related enzymes
        - 1 Fumarase, crotonase
        - 2. Aldehyde mutases
        - 3. Mutases of ketocarboxylic acids
        - 4 Aspartase
    - (*b*) Carboxylases
    - (*c*) Carbonic anhydrase
    - (*d*) Enzymes of the "first attack" on sugars (aldolase, isomerase, carboligase (?))
    - (*e*) Peroxidases (classical peroxidase, cytochrome peroxidase, verdoperoxidase, etc.)
    - (*f*) Catalases
    - (*g*) Reductases (azotases, hydrogenases)

Selected Bibliography

Bamann, E , and Myrbaeck, K , *Die Methoden der Fermentforschung* Thieme, Leipzig, 1940

Green, D E , *Mechanisms of Biological Oxidations* Macmillan, New York, 1940

Haldane, J B S , *Enzymes* Longmans, Green, London, 1930, Haldane, J B S , and Stern, K G , *Allgemeine Chemie der Enzyme* Steinkopff, Dresden, 1932

Nord, F F , and Weidenhagen, R , *Handbuch der Enzymologie* Akademische Verlagsgesellschaft, Leipzig, 1940, Edward Bros reprint, Ann Arbor, 1943

Northrop, J H , *Crystalline Enzymes* Columbia Univ Press, New York, 1939

Oppenheimer, C , and Kuhn, R , *Die Fermente und ihre Wirkungen* Thieme, Leipzig, 1925, Oppenheimer, C , *Supplement*, Junk, Amsterdam, 1936–1939

Oppenheimer, C , and Pincussen, L , *Methodik der Fermente* Thieme, Leipzig, 1929

Oppenheimer, C , and Stern, K G , *Biological Oxidation* Junk, Amsterdam, 1939

Rona, R , *Praktikum der Physiologischen Chemie*, Vol I Fermentmethoden Springer, Berlin, 1925.

Sumner, J B , and Somers, G F , *Chemistry and Methods of Enzymes.* Academic Press, New York, 1943

Tauber, H., *Enzyme Chemistry.* Wiley, New York, 1937.

Chapter VII

VITAMINS, VITAGENS, AND HORMONES

H. R. Rosenberg

Wilmington, Delaware

Vitamins, vitagens, and hormones have in common the property of being organic substances which perform, in the organism, specific and vital functions in relatively small concentrations.

Vitamins are defined[1] as organic compounds which are required for the normal growth and maintenance of life of animals, including man, who, as a rule, are unable to synthesize these compounds by anabolic processes that are independent of environment other than air, and which compounds are effective in small amounts, do not furnish energy, and are not utilized as building units for the structure of the organism, but are essential for the transformation of energy and for the regulation of the metabolism of structural units.

Vitagens are differentiated[1] from vitamins according to the function of these compounds in the organism. Thus the vitagens are essential not only as transformers of energy or regulators of the metabolism of structural units, but also as suppliers of energy or as structural building units.

Hormones are organic compounds which differ from vitamins in that they are produced within the organism. In all other respects, the hormones conform to the vitamin definition.

In the absence of vitamins, vitagens, or hormones, clinical deficiency symptoms occur sooner or later. Generally speaking, the well-being of a person suffers in any such deficiency state. The growing organism does not continue its normal development, and in the adult organism an early aging sets in. Each deficiency is usually characterized by the occurrence of specific symptoms.

I. THE VITAMINS[1]

According to the customary manner and following in principle the historical development, the vitamins will be discussed in the alphabetical order of nomenclature. The knowledge of the function of the vitamins has not as yet advanced enough to permit a classification of the vitamins according to their action.

There are 14 vitamins, or in some cases groups of vitamins, which have been characterized as chemical entities and which perform specific functions. There is, first, vitamin A and its various naturally occurring provitamins. There is then what has been called the vitamin B complex which comprises, besides some less well-defined compounds, vitamin B_1 or thiamine, vitamin B_2 or riboflavin, vitamin B_6 or pyridoxine, nicotinamide, pantothenic acid, inositol, and *p*-aminobenzoic acid. These B vitamins are followed by vitamin C, the group of vitamins D and their provitamins, the group of vitamins E, vitamin H or biotin which can also be classified as a member of the vitamin B complex, the group of vitamins K and vitamin P. In addition to these well-known chemical compounds, there are a considerable number of less well-defined compounds which are summarized in a paragraph on the nonidentified vitamins.

---

[1] H. R. Rosenberg, *Chemistry and Physiology of the Vitamins.* Interscience, New York, 1942.

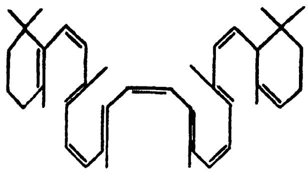
1. The Group of Vitamins A

The group of vitamins A is subclassified into provitamins A and vitamin A. The provitamins A are compounds of twice as many carbon atoms as vitamin A and are not known to exert any vitamin A activity of their own. They are converted in the organism into the smaller molecule of the vitamin A structure.

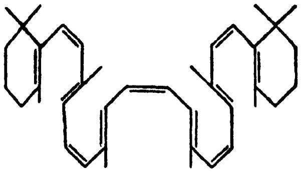
(*a*) *Provitamins A*

There are at least nine different chemical compounds known as provitamins A, namely, α-, β-, γ-, and *K*-carotene, cryptoxanthene, echinenone, myxoxanthin, aphanin, and aphanicin. There is the definite possibility that other naturally occurring compounds of the same class may be provitamins A. Thus leprotene may be a provitamin A although this has not been definitely established. Of these nine different provitamins A only five, namely, the carotenes and cryptoxanthene, are known to occur in the normal food of man.

The various provitamins A have the following chemical structures:[2]



α-Carotene (α,β-Carotene): M. p. 187°. $[\alpha]_{844} = +323°$. Absorption maxima at 511, 478, and 452 mμ (CS_2).

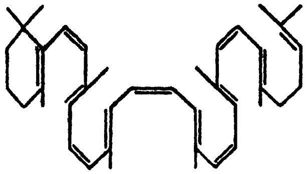


β-Carotene (β,β-Carotene): M.p. 184°. Absorption maxima at 520, 484, and 452 mμ (CS_2).

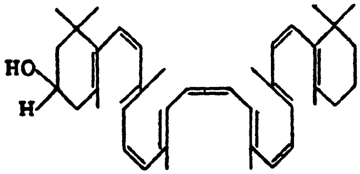
---

[2] P. Karrer and coworkers, *Helv. Chim. Acta*, **13**, 1084 (1930); **14**, 1033 (1931). R. Kuhn and H. Brockmann, *Ann.*, **516**, 95 (1935).

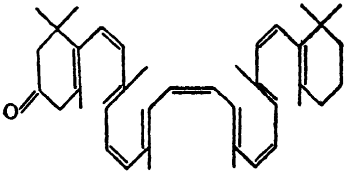
γ-Carotene (β-Lyco-β-carotene): M. p. 178°. Absorption maxima at 533, 496, and 463 mμ (CS_2).



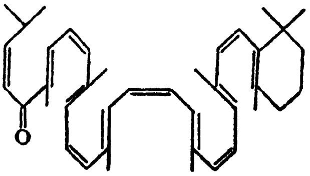
Cryptoxanthene (3-Hydroxy-β,β-carotene): M. p. 169°. Absorption maxima at 520, 484, and 452 mμ (CS_2).



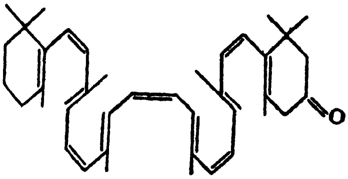
Echinenone: Structure questionable. M. p. 192–193°. Absorption maxima at 520, 488, and 450 mμ (CS_2).



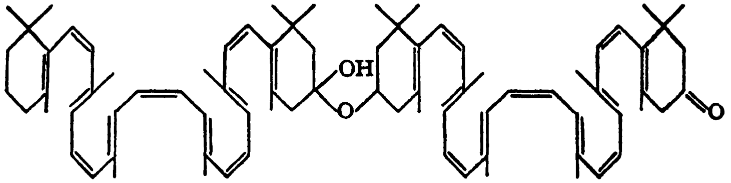
Myxoxanthin: M. p. 168–169°. Absorption maximum at 488 mμ (CS_2).



Aphanin: Structure questionable. M. p. 180°. Absorption maxima at 533.5 and 494 mμ (CS_2).

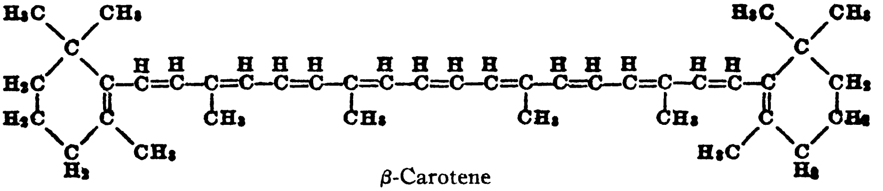


Aphanicin: Structure questionable. M. p. 195°. Absorption maxima at 533 and 494 mμ (CS_2).



The constitution of *K*-carotene has not been established.[3]

The aliphatic part of the molecule is written in the manner of Ruzicka in the form of "open rings." This formula is not intended to indicate the true spatial arrangement of the aliphatic chain, but to stress the relations to similar compounds and to impress the memory. Many authors prefer the use of the "long-chain" formula:


β-Carotene

The provitamins A crystallize in deep red prisms of high melting points and exhibit typical absorption spectra, the positions of the maxima of which change with different solvents. α-Carotene is the only provitamin A with an asymmetric carbon atom and is, therefore, the only optically active provitamin A.

All provitamins A have similar solubility characteristics. They dissolve readily in chloroform, carbon disulfide, and benzene, and more difficultly in petroleum ether. They are insoluble in water and practically insoluble in alcohol. Cryptoxanthene, because of the presence of a hydroxyl group, is somewhat soluble in alcohol. All provitamins A are soluble in fats.

The provitamins A are extremely sensitive to oxidation, autoxidation, and light. They are stable to heat in an inert atmosphere.

The provitamins A occur in nature only to a small extent in the free form. They are mainly bound in complex form to proteins. Cryptoxanthene occurs predominantly in ester form.

(*b*) *Conversion of Provitamins A to Vitamin A*

The conversion of provitamins A into vitamin A is carried out in the organism, probably in the liver. The mechanism of this reaction is not known. While it may appear possible that β-carotene yields two molecules of vitamin A, whereas all other provitamins A can yield only one molecule of this vitamin, experience has shown that even under optimum conditions an asymmetrical fission of the provitamin molecule occurs.[4] Consequently, β-carotene yields a maximum of only one molecule of vitamin A, while at least two molecules of the other provitamins are required for the formation of one vitamin A molecule. Under normal conditions, the yield of vitamin A is much smaller.

---

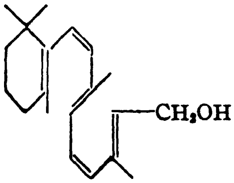
[3] G. S. Fraps and A. R. Kemmerer, *Ind. Eng. Chem., Anal. Ed.*, **13**, 806 (1941).

Not all animals are able to convert provitamins A into vitamin A with the same efficiency. While the human organism is apparently able to convert the carotenes into vitamin A, there are indications that human beings may have difficulty in utilizing cryptoxanthene as a source of vitamin A.[5] Rats possess the most efficient known system of conversion. Chickens, guinea pigs, rabbits, pigs, and cattle are less efficient and carnivorous animals are able to utilize provitamins A only to a very limited extent.

The conversion of provitamins A to vitamin A is a specific physiological process which cannot be duplicated by laboratory means.

(c) *Vitamin A*

Vitamin A, for which the names *carenol* and *axerophthol* have been suggested in America and Europe, respectively, has the empirical formula $C_{20}H_{30}O$ and has the following chemical structure:[6]



The crystallized vitamin A has a biological activity of 4,500,000 International Units (I. U.) per gram.[7] One I. U. is equivalent to one U. S. P. Unit and is based upon the biological response of 0.6 γ of pure β-carotene under well-defined conditions.

Vitamin A crystallizes in pale yellow plates, melting at 63–64° C., from ethyl formate or propylene glycol. From methanol, vitamin A crystallizes with solvent of crystallization and melts at 8° C.

Vitamin A distills under the conditions of molecular distillation at 10^{-3} mm. at 120–125° C. The absorption spectrum of vitamin A in the ultraviolet shows a sharp band at 328 mμ, with an extinction coefficient $E^{1\%}_{1\,\mathrm{cm.}} = 1750$ for the crystalline vitamin.

Vitamin A is sensitive to oxidation and autoxidizes readily. It is heat-stable in an inert atmosphere and is stable when dissolved in oils from natural sources. It is soluble in most organic solvents and is insoluble in water.

---

[4] S. W. F. Underhill and K. H. Coward, *Biochem. J.*, **33**, 589 (1939).

[5] A. G. Van Veen and J. C. Lanzing, *Proc. Acad. Sci. Amsterdam*, **40**, 779 (1938).

[6] P. Karrer, R. Morf, and K. Schöpp, *Helv. Chim. Acta*, **14**, 1431 (1931); **16**, 557, 625 (1933).

[7] J. G. Baxter, P. L. Harris, K. C. D. Hickman, and G. D. Robeson, *J. Biol. Chem.*, **141**, 991 (1941).

Vitamin A occurs only in the animal organism. It has not been found in plants. Usually about 10 to 30% of the total vitamin A is present in the form of the free alcohol, while the remainder is present as esters, chiefly palmitic, accompanied by esters of C_{14} and C_{20} acids. The most important technical method of isolating vitamin A from fish oils, the main source of this vitamin, is by molecular distillation. Vitamin A is not available by synthesis although a procedure is known by which small amounts of an active but impure material can be obtained.

Besides vitamin A, there exist other chemically related compounds which exert a certain amount of vitamin A activity. Relatively little is known about these other vitamin A factors. The so-called vitamin A_2 appears to have an activity of about 45,000 U. S. P. units per g.[8] and occurs in certain fresh-water fish. Another compound of vitamin A activity is the anhydro-vitamin A which occurs in traces in all vitamin A-bearing oils and which is reported to have an activity of about 16,000 U. S. P. units per g.

Kitol is the name given to a compound isolated from whale liver oil. Although this compound has no vitamin A activity, thermal decomposition transforms kitol into a vitamin A active compound. Chemically, kitol appears to be a divitamin A containing two hydroxyl groups.

The optimum requirements for an average adult are about 5000 I. U. of vitamin A or about 15,000 I. U. of provitamin A. These double standards must be recognized since the I. U. of vitamin A and of provitamin A are equal only under the specified conditions of the biological test, but not in the metabolism of a normal organism. The vitamin A requirements of adult mammals can be correlated to body weight. About 12 γ or 60 I. U. of vitamin A or 125 γ or 200 I. U. of provitamin A are considered an optimum daily dosage per kg. of body weight.

The action of vitamin A can be regarded essentially as a stimulus for the formation of new cells. This vitamin is necessary to maintain the normal epithelial tissues of the body and a lack of this vitamin causes keratinization which is observed, for example, on the conjunctiva of the eye, the respiratory passages, or the intestinal tract. Dryness of the skin and scaliness of the hair follicles are observed. Deficiency of vitamin A is accompanied by a lowered resistance to infection. This vitamin is also essential for the proper formation of tooth enamel. A characteristic deficiency symptom is impaired vision which results in the so-called night blindness.

The mechanism of the vitamin A action in the organism is not known.

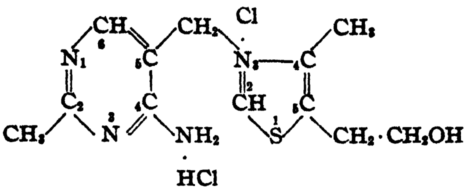
2. Vitamin B_1—Thiamine[9]

Vitamin B_1 is called *thiamine* in America, *aneurin* in Europe, and *oryzanine* in Japan. It is a base and is generally utilized as the chloride-hydrochloride. It has the empirical formula $C_{12}H_{17}ON_4SCl \cdot HCl$ and has the following chemical structure:

---

[8] J. L. Jensen, E. M. Shantz, N. D. Embree, J. D. Cawley, and P. L. Harris, *J. Biol. Chem.*, **149**, 473 (1943).

[9] R. R. Williams and T. D. Spies, *Vitamin B_1*. Macmillan, New York, 1938.



Vitamin B_1-chloride-hydrochloride has a biological activity of 333,000 I. U. (or U. S. P. Units) per gram. One I. U. is equal to 3 γ of the pure crystalline vitamin.

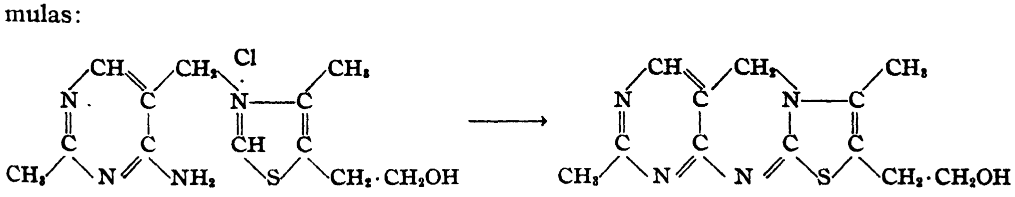
Vitamin B_1 crystals and solutions have a distinct yeastlike or nutty odor, which is easily recognized.

Vitamin B_1 crystallizes in colorless needles as the hemihydrate from alcoholic aqueous solution and melts at 248–250° C. with decomposition. Commercial vitamin B_1 contains about 4% water which can be removed by drying at 100° C. or by standing in a vacuum over sulfuric acid. On exposure to air of average humidity vitamin B_1 crystals absorb up to one mole of water.

Vitamin B_1 is amorphous as the free base. Besides the hydrochloride many other salts have been prepared. The hydrochloride is insoluble in the usual organic solvents such as ether, chloroform, benzene, and acetone. It is soluble in water to the extent of 1 g. per ml. In 95% alcohol 1 g. dissolves in 100 ml. while in absolute alcohol 315 ml. are necessary to dissolve this quantity.

Vitamin B_1 exhibits a characteristic absorption spectrum in the ultraviolet. The positions of the maxima are a function of the hydrogen-ion concentration. At *p*H 7 (or higher) two bands at 235 and 267 mμ, respectively, are observed, while only one band, at 245 to 247 mμ, is noted at *p*H 5.5 (or less).

Vitamin B_1 is stable in strongly acidic solution. The hydrochloride can be heated to 120° C. at *p*H 3.5 without decomposition. In weakly acidic solution, however, the molecule undergoes a cleavage and the biological activity disappears. In neutral and alkaline solution, vitamin B_1 is extremely sensitive to heat. Vitamin B_1 is also readily destroyed by oxidation and reduction. Oxidation yields *thiochrome*, a yellow substance of intense blue fluorescence. This conversion and the outstanding properties of thiochrome have been used as the basis of a method for the determination of vitamin B_1. The conversion of the vitamin to thiochrome is evident from the structural formulas:



Vitamin B_1 Thiochrome

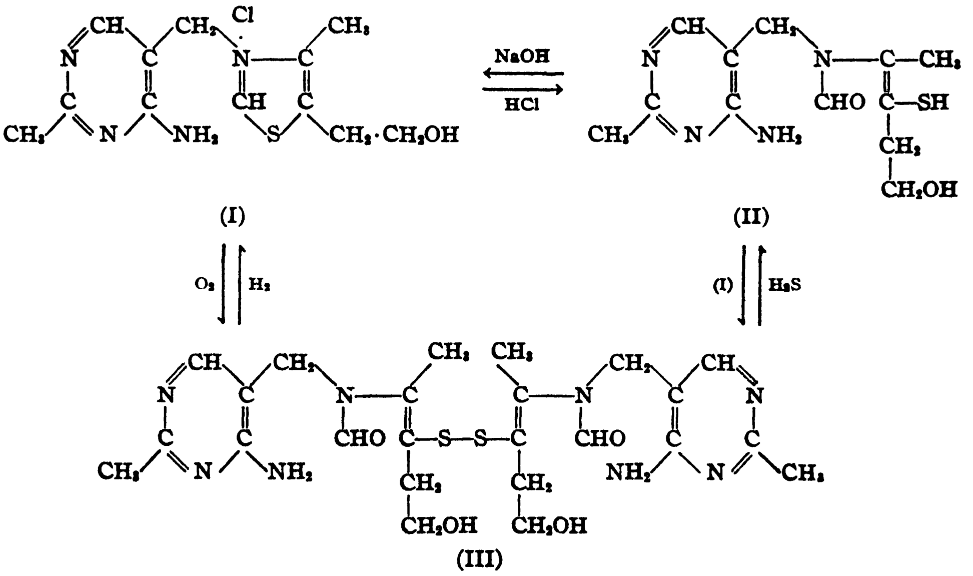
Vitamin B_1 is widely distributed over the plant kingdom. Most leaves contain about 25 I. U. per 100 g. It occurs in high concentration in seeds, especially in outside bran coats. It is present in small amounts throughout the normal animal organism, but is not stored. Pork muscles, however, contain about eight times as much as beef muscles.

Vitamin B_1 occurs as the free compound, as a vitamin B_1-protein complex, as vitamin B_1-pyrophosphate which is known as the important coenzyme cocarboxylase, and as a vitamin B_1-phosphorus-protein complex. The relative amounts of these forms vary considerably in different sources.

Chemically, vitamin B_1 is a compound which contains two ring systems, a pyrimidine ring and a thiazole ring, which are connected with each other through a methylene bridge. The pyrimidine ring is substituted by a methyl group in 2-position and an amino group in 4-position. The bridge to the thiazole ring is connected through carbon atom 5 and joins the thiazole through the nitrogen in 3-position, thus forming a quaternary ammonium compound. The thiazole is further substituted by a methyl group in 4-position and a β-hydroxyethyl group in 5-position. Vitamin B_1 can be synthesized by a number of different procedures and the commercially available pure material originates from syntheses exclusively.

The human requirements for vitamin B_1 are in the order of 1–3 mg. per day and are a function of the type of food supplied (carbohydrates increase, fats decrease the amount of vitamin B_1 needed); of the intensity of the metabolism; of the outside temperature and of other factors.

Vitamin B_1 probably represents the reduced form of an oxidation-reduction system.[10] Under physiological conditions it can be oxidized to a disulfide which in turn can be reduced to the vitamin through the thiol (II). In the organism, the reduction apparently does not stop at the thiol stage. The disulfide (III) exhibits the full biological activity of vitamin B_1 and is about five times less toxic than the vitamin.



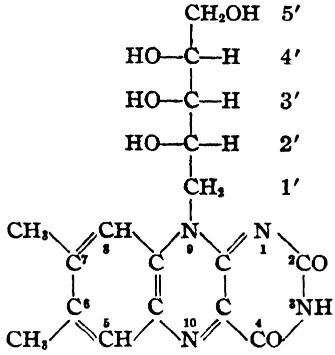
---

[10] O. Zima and R. R. Williams, *Ber.*, 73, 941 (1940).

Vitamin B_1 is essential for the proper utilization of carbohydrates. A deficiency of this vitamin affects first the emotions and the nervous system. Loss of appetite, unusual susceptibility to fatigue, and gastrointestinal disturbances are common. The diseases of advanced vitamin B_1 deficiency are known as *polyneuritis* and *beriberi* which are caused by injuries to nerve tissues and which manifest themselves by such symptoms as lameness, disturbances of the motor and sensory nerves, hypertrophy of the heart, and finally death from heart failure.

3. Vitamin B_2—Riboflavin

Vitamin B_2 is called *riboflavin* in America and *lactoflavin* in Europe. It has the empirical formula $C_{17}H_{20}N_4O_6$ and has the following chemical structure:[11]



Vitamin B_2 has a biological efficacy of 400,000 B. S. U. (Bourquin-Sherman Units) per gram. One B. S. U. is defined as that amount of the vitamin which insures a certain amount of growth under certain conditions. The activity of vitamin B_2 is thus defined by biological units which refer to the reaction of an animal and not by a weight standard which refers to the amount of the vitamin. The proposed I. U. is 5 γ of the pure, crystalline vitamin.

Riboflavin crystallizes in fine orange-yellow needles which begin to darken at about 240° C. and melt at 282° C. with decomposition. The pure vitamin is soluble in water to the extent of 12 mg. and in alcohol to the extent of 4.5 mg. in 100 ml. at 27.5° C. It is also slightly soluble in such organic solvents as amyl alcohol, cyclohexanol, phenol, and amyl acetate, but is practically insoluble in acetone, ether, benzene, and chloroform. Vitamin B_2 is very soluble in alkaline solutions.

A water solution of riboflavin is greenish yellow in color and displays an intense yellow-green fluorescence which vanishes on the addition of acids or alkali. The characteristic fluorescence spectrum with a maximum at 565 $m\mu$ is used for the quantitative determination of the vitamin. Optimum fluorescence occurs at *p*H 3 to 9. The isoelectric point of riboflavin is at *p*H 6. The isoelectric constants of the amphoteric riboflavin have been calculated as: $K_{acid} = 63 \times 10^{-12}$ and $K_{base} = 0.5 \times 10^{-5}$. Riboflavin shows optical rotation: $[\alpha]_D^{20} = -114°$ in 0.1 N sodium hydroxide solution. In neutral and in acid solution, the optical activity is exceedingly small. Riboflavin has a characteristic absorption spectrum with maxima at 445, 372, 269, and 225 mμ.

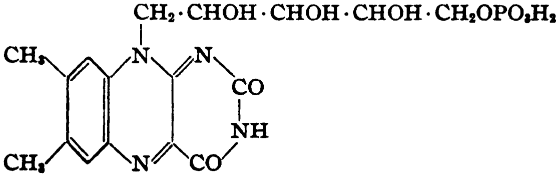
---

[11] P. Karrer and coworkers, *Helv. Chim. Acta*, **18**, 426, 522, and 1435 (1935).

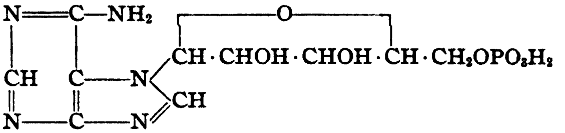
Crystalline riboflavin, when protected against light, is stable at ordinary temperatures. In solution, riboflavin is unstable. The decomposition is greatly accelerated in alkaline solution and in the presence of light. Riboflavin possesses a relatively high degree of thermostability and only slight destruction occurs by heating at 120° C. for 6 hrs.

Chemically, riboflavin is a dimethyl derivative of isoalloxazine and has a *d*-ribose (see page 68) side chain attached to the nitrogen in 9-position. Riboflavin has been synthesized by a number of different procedures some of which are used commercially.

Riboflavin is widely distributed over the entire animal and plant kingdom, but occurs only in very small concentrations. In places where no respiration or fermentation occurs, the free riboflavin is found. Otherwise the free form exists only to a small extent while the greater part occurs as the 5′-phosphoric acid ester (I) or as riboflavin-phosphoric acid-adenine-dinucleotide, which is composed of the riboflavin-5′-phosphoric acid (I) and adenylic acid (adenosine -5′-monophosphoric acid, II).[12]



(I)



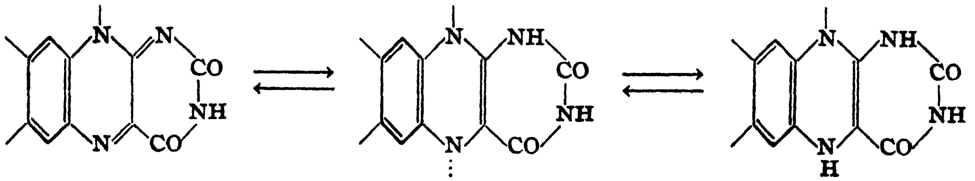
(II)

Man needs 2 to 3 mg. of riboflavin daily. Generally, the vitamin B_2 requirements of animals are related to body size and weight, to the amount and type of food ingested, to the ambient temperature, etc.

The activity of riboflavin is attributed to its participation in various enzyme systems. The mechanism of this action consists in reversibly accepting and donating two atoms of hydrogen. This is accomplished by the addition of hydrogen to the 9- and 10-positions of riboflavin. This reversible oxidation-reduction probably goes through the quinhydrone stage.

---

[12] O. Warburg and W. Christian, *Biochem. Z.*, **298**, 150, 368 (1938).



Riboflavin (yellow)

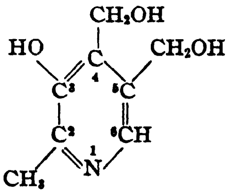
Chloroflavin (green) (quinhydrone)

Leucoflavin (colorless)

Riboflavin is essential in the metabolism of carbohydrates. A deficiency of this vitamin results in impaired growth. Specifically *cheilosis*, a cracking of the lips and the corners of the mouth, is observed. There is also an accumulation of a greasy material at the angles of the nose. In addition, there are some characteristic changes in the eye which begin with an itching sensation and a mild photophobia. The most characteristic change in the eye is the vascular invasion of the cornea.

4. Vitamin B_6—Pyridoxine

Vitamin B_6 is called *pyridoxine* in America and *adermin* in Europe. It has the empirical formula $C_8H_{11}O_3N$ and has the following chemical structure:[18]



Pyridoxine has a biological efficacy of 100,000 rat units per gram. One rat unit is the daily minimum dosage required by rats and is equivalent to $10\ \gamma$. No other standard has been established.

Vitamin B_6, as the free base, is a colorless crystalline powder, has a slightly bitter taste, and melts at 160° C. It is readily soluble in water, in acetone, and in alcohol, and slightly soluble in ether and chloroform. It readily forms salts, such as the hydrochloride, m. p. 204–206° C. (with decomposition). The hydrochloride is soluble in water to the extent of 1 g. in 4.5 ml. and in alcohol to the extent of 1 g. in 90 ml. The aqueous solution of the hydrochloride has a *p*H of about 3.2. Both the free vitamin and the hydrochloride sublime readily. The hydrochloride is the form in which this vitamin is normally marketed. It is a white, odorless powder with salty taste. It is stable to heat, concentrated hydrochloric acid and alkali, but is destroyed by light.

Vitamin B_6 is optically inactive. It exhibits a typical ultraviolet absorption spectrum which changes markedly with a change of the hydrogen-ion concentration. In strongly acidic solution, there is a single band at 292 mμ. Between *p*H 4 and 5 two new bands appear at 255 and 326 mμ. As the alkalinity increases the 292 mμ band disappears and the 326 mμ band becomes more intense. There is in addition a shift toward the shorter wave lengths with increasing alkalinity. The maxima are finally observed at 246 and 311 mμ.

---

[18] E. T. Stiller, J. C. Keresztesy, and J. R. Stevens, *J. Am. Chem. Soc.*, **61**, 1237 (1939). R. Kuhn, G. Wendt, and K. Westphal, *Ber.*, **72**, 310 (1939).

Chemically, pyridoxine is a pyridine derivative, substituted in 2-position by a methyl group, in 3-position by a phenolic hydroxyl group and in 4- and 5-position by methylol groups. It has been synthesized by various routes and the synthetic material is commercially available.

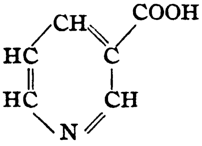
Pyridoxine appears to be widely distributed in both plants and animals. Seeds, yeast, and livers contain relatively high concentrations. It occurs in animal and in plant tissue only to a small extent in the free form. About 60 to 80% is chemically bound to protein and to starch.

The exact human requirement of pyridoxine has not been established, but is believed to be of the order of 0.1 to 1.0 mg. The action and the reaction mechanism of this vitamin are not fully understood.

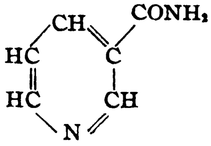
A deficiency of vitamin B_6 in man has not been associated so far with specific clinical symtoms. In certain cases of pellagra, vitamin B_6 deficiency appeared to be responsible for nervousness, insomnia, cramping pains in the stomach, and muscular weakness and rigidity. In animals fits of an epileptic appearance are observed. In some cases death through spastic convulsions has been noted in animals. There is also a specific type of dermatitis which is characteristic in certain animals.

5. Nicotinic Acid and Nicotinamide[14]

Nicotinic acid and nicotinamide are called *niacin* and *niacin amide.* They have the empirical formulas $C_6H_5O_2N$ and $C_6H_6ON_2$, respectively. They have the following chemical structures:



Nicotinic acid



Nicotinamide

There is no unit or standard for nicotinic acid or nicotinamide. These compounds are used on the weight basis exclusively.

Free nicotinic acid apparently does not occur in the living organism. It is found, however, in the urine of animals. Nicotinic acid occurs in tissues in the form of the amide. Since human beings and, in general, animal organisms are capable of amidating nicotinic acid, it is often referred to as the vitamin. Foods actually contain only nicotinamide, which is the only compound that should be called a vitamin. The use of the amide is preferable from a pharmacological standpoint, since the free acid causes certain toxic reactions.

---

[14] C. A. Elvehjem, *Physiol. Revs.*, **20**, 249 (1940).

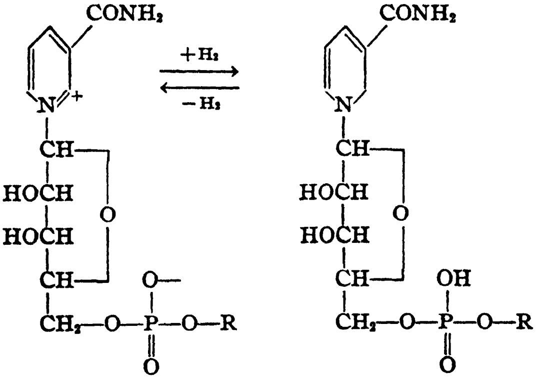
Nicotinic acid crystallizes in needles from water or alcohol and melts at 235.5–236.5° C. It sublimes without decomposition. Nicotinic acid exhibits a typical absorption spectrum with a maximum at 385 $m\mu$.

Nicotinamide crystallizes in needles from benzene and melts at 129°. It distills at 150–160° C. and 10^{-4} mm. Hg.

Chemically, nicotinic acid is pyridine-3-carboxylic acid. Nicotinamide is the corresponding acid amide. Nicotinic acid has been synthesized and is prepared commercially by degradation of alkylated pyridines, such as nicotine.

The optimum human requirement of nicotinamide has been estimated at about 10 to 25 mg. per day.

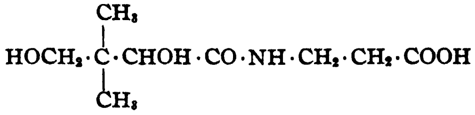
The activity of nicotinamide is due to the part it plays in a number of different dehydrogenation enzyme systems. In this action, the nicotinamide part of the enzyme absorbs two atoms of hydrogen, thus becoming a dihydronicotinamide, which is easily reconverted into the original nicotinamide. This reaction does not occur with nicotinamide itself, but with derivatives, such as the dehydrogenases, in which the ring-nitrogen of nicotinamide is chemically bound in such a way that upon reduction a tertiary nitrogen is formed. Upon oxidation a quaternary pyridinium salt is obtained. The mechanism of the nicotinamide in the dehydrogenases is illustrated by the following formulas:



Nicotinamide is essential in the metabolism of carbohydrates. The typical symptoms of severe nicotinamide deficiency are summarized in the term *pellagra*. This disease is characterized by lesions of the mucous membranes, for example in the mouth, and of the skin over the nose, forehead, wrists, hands, knees, and feet. Furthermore, there are disturbances of the gastrointestinal tract. In the more advanced stages of the disease, mental disorders and a clouding of the consciousness are common and lead to death through insanity.

6. Pantothenic Acid[15]

Pantothenic acid, for which the abbreviated term *pantothen* has been suggested, may be identical with the postulated vitamin B_3. It has the empirical formula $C_9H_{17}O_5N$ and is of the following chemical structure:[16]



$$HOCH_2\cdot\overset{CH_3}{\underset{CH_3}{C}}\cdot CHOH\cdot CO\cdot NH\cdot CH_2\cdot CH_2\cdot COOH$$

Pantothenic acid is generally used on the weight basis. The sodium salt has been suggested as standard but the calcium salt is the form most commonly offered for sale. Several biological units such as the chick unit (14 γ), the yeast unit, etc., are still encountered occasionally.

Pantothenic acid in pure form is a pale yellow viscous oil. The vitamin is dextrorotatory, $[\alpha]^{25}_{D} = +37.5°$. The calcium salt is of microcrystalline structure, $[\alpha]^{26}_{D} = +24.3°$.

Pantothenic acid is an amphoteric substance but is predominantly of acid character. The vitamin is readily soluble in water, ethyl acetate, dioxane, and acetic acid; is somewhat soluble in ether and amyl alcohol; and is practically insoluble in benzene and chloroform.

Chemically, pantothenic acid is an aliphatic acid and an *N*-substituted acid amide. α,γ-Dihydroxy-β,β-dimethylbutyric acid is bound in acid amide form to β-alanine. Pantothenic acid has been synthesized and the synthetic racemic product has been resolved into its optical isomers.

Pantothenic acid occurs in all types of animal tissues and seems to be present universally in protoplasm. In animal tissue (and probably also in plant tissue) pantothenic acid occurs free only to a very small extent, but is mainly bound to protein material.

Pantothenic acid is undoubtedly essential for man, although the requirements have not been established. It is assumed that the optimum need is well satisfied by 1 mg. of calcium pantothenate.

Pantothenic acid is believed to be involved in the carbohydrate metabolism. The mechanism of this action is not known.

There are no deficiency symptoms known to occur when pantothenic acid is absent from the diet of man. Chicks suffer from incrustations about the eyes, in the corners of the mouth, and in areas between the toes. In fur-bearing animals a graying of the hair is observed in many cases. Throughout the animal kingdom there is also an impairment of growth and reproduction.

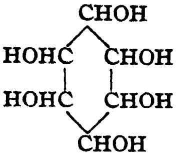
---

[15] R. J. Williams, *Enzymologia*, **9**, 387 (1941).

[16] R. J. Williams, H. H. Weinstock, E. Rohrmann, J. H. Truesdail, H. K. Mitchell, and C. E. Meyer, *J. Am. Chem. Soc.*, **61**, 454 (1939). E. T. Stiller, S. A. Harris, J. Finkelstein, J. C. Keresztesy, and K. Folkers, *ibid.*, **62**, 1785 (1940).

7. Inositol[17]

Inositol has long been known as one of the bios factors. It has the empirical formula $C_6H_{12}O_6$ and has the chemical formula of hexahydroxy-cyclo-hexane:



There is no standard or biological unit for inositol. Inositol has a sweet taste. Its dihydrate melts at 215–216° C., while the anhydrous compound melts at 225–226° C. It is soluble in water to the extent of one part in 5.7 parts of water at 24° C., but is insoluble in absolute alcohol and in ether. It crystallizes from acetic acid or from water above 50° C. as the water-free compound, but below 50° C. as the dihydrate.

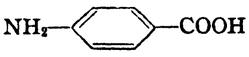
Inositol occurs in animal tissues predominantly in an alkali-labile combination with a large molecule, probably a protein. Some inositol appears to occur in the free state. In plants, most of it is present in the form of the hexaphosphate which is known as *phytic acid.* In some plant materials inositol is bound in the phosphatide fraction as a glucoside.

The human requirements of inositol have not been established. The physiological action is essentially unknown.

Inositol deficiency symptoms are known only for mice and rats. Mice become completely hairless over the trunk. In advanced conditions severe dermatitis occurs. In rats the denudation sets in around the eyes.

8. *p*-Aminobenzoic Acid[18]

p-Aminobenzoic acid has been postulated as a member of the vitamin B complex. It has the empirical formula $C_7H_7O_2N$ and has the following chemical structure:



p-Aminobenzoic acid is a colorless substance which melts at 186–187° C. It is apparently widely distributed over the entire plant and animal kingdom.

This vitamin is required as a growth factor by many bacteria. The human requirements have not been definitely established. In human therapy a dose of 100–200 mg. daily has been used. The mechanism of its activity is not known.

A deficiency in *p*-aminobenzoic acid causes a graying of the fur of the black rat. There is also a retardation of growth. There are indications that *p*-aminobenzoic acid may be one of many factors necessary for the maintenance of the hair pigment in man.

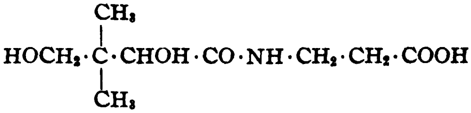
---

[17] D. W. Woolley, *Science*, **92**, 384 (1940).

[18] S. Ansbacher, *Science*, **93**, 164 (1941). B. F. Sieve, *ibid.*, **94**, 257 (1941).

6. Pantothenic Acid[15]

Pantothenic acid, for which the abbreviated term *pantothen* has been suggested, may be identical with the postulated vitamin B_3. It has the empirical formula $C_9H_{17}O_5N$ and is of the following chemical structure:[16]



$$\mathrm{HOCH_2\cdot\overset{\displaystyle CH_3}{\underset{\displaystyle CH_3}{C}}\cdot CHOH\cdot CO\cdot NH\cdot CH_2\cdot CH_2\cdot COOH}$$

Pantothenic acid is generally used on the weight basis. The sodium salt has been suggested as standard but the calcium salt is the form most commonly offered for sale. Several biological units such as the chick unit (14 γ), the yeast unit, etc., are still encountered occasionally.

Pantothenic acid in pure form is a pale yellow viscous oil. The vitamin is dextrorotatory, $[\alpha]_D^{25} = +37.5°$. The calcium salt is of microcrystalline structure, $[\alpha]_D^{26} = +24.3°$.

Pantothenic acid is an amphoteric substance but is predominantly of acid character. The vitamin is readily soluble in water, ethyl acetate, dioxane, and acetic acid; is somewhat soluble in ether and amyl alcohol; and is practically insoluble in benzene and chloroform.

Chemically, pantothenic acid is an aliphatic acid and an *N*-substituted acid amide. α,γ-Dihydroxy-β,β-dimethylbutyric acid is bound in acid amide form to β-alanine. Pantothenic acid has been synthesized and the synthetic racemic product has been resolved into its optical isomers.

Pantothenic acid occurs in all types of animal tissues and seems to be present universally in protoplasm. In animal tissue (and probably also in plant tissue) pantothenic acid occurs free only to a very small extent, but is mainly bound to protein material.

Pantothenic acid is undoubtedly essential for man, although the requirements have not been established. It is assumed that the optimum need is well satisfied by 1 mg. of calcium pantothenate.

Pantothenic acid is believed to be involved in the carbohydrate metabolism. The mechanism of this action is not known.

There are no deficiency symptoms known to occur when pantothenic acid is absent from the diet of man. Chicks suffer from incrustations about the eyes, in the corners of the mouth, and in areas between the toes. In fur-bearing animals a graying of the hair is observed in many cases. Throughout the animal kingdom there is also an impairment of growth and reproduction.

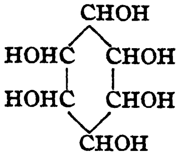
---

[15] R. J. Williams, *Enzymologia*, **9**, 387 (1941).

[16] R. J. Williams, H. H. Weinstock, E. Rohrmann, J. H. Truesdail, H. K. Mitchell, and C. E. Meyer, *J. Am. Chem. Soc.*, **61**, 454 (1939). E. T. Stiller, S. A. Harris, J. Finkelstein, J. C. Keresztesv, and K. Folkers, *ibid.*, **62**, 1785 (1940).

7. Inositol[17]

Inositol has long been known as one of the bios factors. It has the empirical formula $C_6H_{12}O_6$ and has the chemical formula of hexahydroxy-cyclo-hexane:



There is no standard or biological unit for inositol. Inositol has a sweet taste. Its dihydrate melts at 215–216° C., while the anhydrous compound melts at 225–226° C. It is soluble in water to the extent of one part in 5.7 parts of water at 24° C., but is insoluble in absolute alcohol and in ether. It crystallizes from acetic acid or from water above 50° C. as the water-free compound, but below 50° C. as the dihydrate.

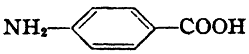
Inositol occurs in animal tissues predominantly in an alkali-labile combination with a large molecule, probably a protein. Some inositol appears to occur in the free state. In plants, most of it is present in the form of the hexaphosphate which is known as *phytic acid.* In some plant materials inositol is bound in the phosphatide fraction as a glucoside.

The human requirements of inositol have not been established. The physiological action is essentially unknown.

Inositol deficiency symptoms are known only for mice and rats. Mice become completely hairless over the trunk. In advanced conditions severe dermatitis occurs. In rats the denudation sets in around the eyes.

8. *p*-Aminobenzoic Acid[18]

p-Aminobenzoic acid has been postulated as a member of the vitamin B complex. It has the empirical formula $C_7H_7O_2N$ and has the following chemical structure·



p-Aminobenzoic acid is a colorless substance which melts at 186–187° C. It is apparently widely distributed over the entire plant and animal kingdom.

This vitamin is required as a growth factor by many bacteria. The human requirements have not been definitely established. In human therapy a dose of 100–200 mg. daily has been used. The mechanism of its activity is not known.

A deficiency in *p*-aminobenzoic acid causes a graying of the fur of the black rat. There is also a retardation of growth. There are indications that *p*-aminobenzoic acid may be one of many factors necessary for the maintenance of the hair pigment in man.

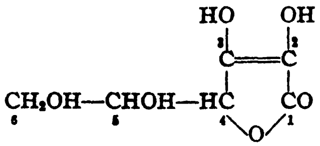
---

[17] D. W. Woolley, *Science*, **92**, 384 (1940).

[18] S. Ansbacher, *Science*, **93**, 164 (1941). B. F. Sieve, *ibid.*, **94**, 257 (1941).

9. Vitamin C—Ascorbic Acid[19]

Vitamin C is called *ascorbic acid*. It has the empirical formula $C_6H_8O_6$ and the following chemical structure:[20]



Vitamin C has a biological activity of 20,000 I. U. (or U. S. P. Units) per gram. One I. U. is equal to 0.05 mg. of the pure ascorbic acid.

Ascorbic acid has a characteristic, somewhat acidic taste. It crystallizes in white, odorless, and colorless plates, melting at 190–192° C. It is soluble in water to the extent of 1 g. in 3 ml. and in absolute alcohol to the extent of 1 g. in 50 ml. It is insoluble in the typical organic fat solvents, such as ether, benzene, chloroform, or fats. Vitamin C is relatively stable in crystalline form, but deteriorates easily in solution. This change is an oxidation and is accelerated by the presence of oxygen (air), traces of metals, especially copper and silver, and light, particularly in the presence of fluorescent materials, such as riboflavin. In the absence of these agents, strongly acidic solutions are stable, but in neutral or alkaline solutions, the vitamin potency is soon lost.

In solution, vitamin C exhibits acidic properties, the dissociation constants being: $pK_1 = 4.17$ and $pK_2 = 11.57$. Ascorbic acid has an optical activity of $[\alpha]_D^{20} = +23°$ in water. It shows a characteristic absorption spectrum in the ultraviolet with a maximum at 265 $m\mu$ and a small band between 350 and 400 $m\mu$.

Chemically, ascorbic acid is not an acid but a lactone forming a five-membered ring of the furan type. The acidic properties of vitamin C are due to the presence of two vicinal enolic hydroxyl groups which are located in the furanoid ring next to the carbonyl of the lactone configuration. Since these hydroxyl groups are enolic, there is a double bond which connects them. The acid, from which the lactone, vitamin C, is derived, is a 2-keto- or 3-ketohexonic acid. Ascorbic acid has been synthesized by a number of different routes, and is commercially available as the synthetic material.

Vitamin C is found in all higher plants in considerable concentration, especially in all growing parts. Citrus fruits, fresh green vegetables, and apples are well-known excellent sources. Vitamin C occurs predominantly in the free form. There is the possibility that this vitamin may also occur in combination with protein material (this combination is called *ascorbigen*), but the existence or nonexistence of ascorbigen has not been definitely established.

The average optimal intake for human beings is about 50–150 mg. of ascorbic acid.

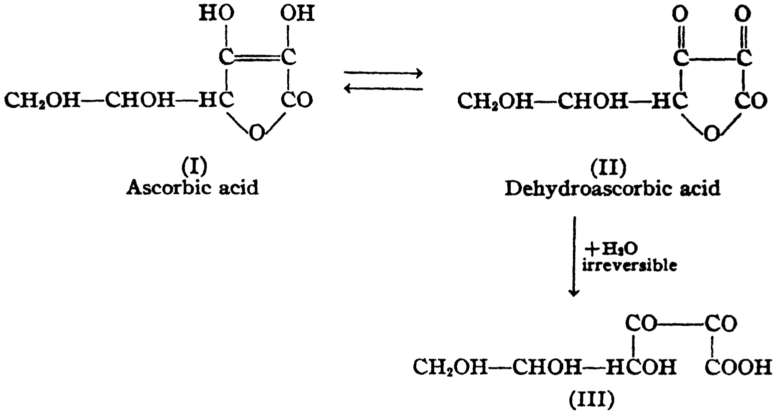
---

[19] C. G. King, *J. Am. Med. Assoc.*, **111**, 1098, 1462 (1938).

[20] W. N. Haworth and E. L. Hirst, *J. Soc. Chem. Ind.*, **52**, 221, 482, 645 (1933). T. Reichstein, A. Grüssner, and R. Oppenauer, *Helv. Chim. Acta*, **16**, 1019 (1933).

The requirements can be calculated approximately on the basis of age, body weight, ambient temperature, etc.

The action of vitamin C in the organism appears to be complex and the mechanism is not well understood. It is postulated that this mechanism depends on the ease with which ascorbic acid (I) undergoes a reversible oxidation to dehydroascorbic acid (II). The latter compound is biologically of the same vitamin activity as the ascorbic acid itself. It can, however, easily undergo a ring-opening (to III) by hydrolysis which is irreversible and which is accompanied by a complete loss of vitamin activity. The oxidation-reduction potential of ascorbic acid at *p*H 4 and at 35° C. is $E'_0 = 0.166\ V$.



(I) Ascorbic acid

(II) Dehydroascorbic acid

(III)

The clinical symptoms of vitamin C deficiency are summarized under the term *scurvy* and are mainly characterized by hemorrhagic conditions. Swollen and bleeding gums are usually the sign of vitamin C deficiency. The site of other hemorrhagic conditions is dependent upon growth and stress. There are also certain changes in the bones which give rise to increased fragility and the dentine in the tooth changes in structure.

10. The Group of Vitamins D[21]

Compounds exerting the physiological effect of vitamins D occur only in the animal organism. Some plants contain similar materials, which are not vitamins D but which can be converted into vitamins D and which are called provitamins D. Provitamins D also occur in some animal tissues.

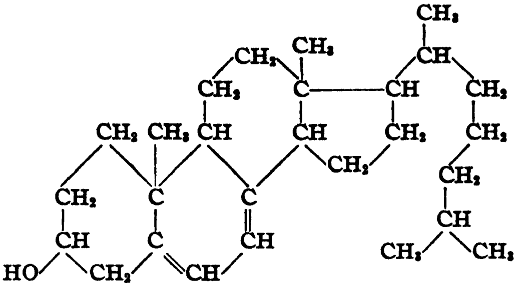
(*a*) *Provitamins D*

There is an unknown number of naturally occurring provitamins D. Only two are definitely identified and fully described, namely, 7-dehydrocholesterol and ergosterol.

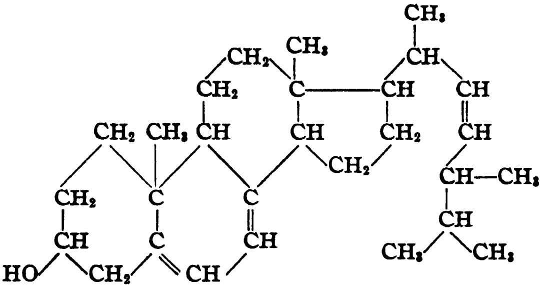
---

[21] H. R. Rosenberg, *Chemistry and Physiology of the Vitamins*. Interscience, New York, 1942. p. 339. C. I. Reed, H. C. Struck, and I. E. Steck, *Vitamin D*. Univ. of Chicago Press, Chicago, 1939,

While the former is the more important provitamin D, both occur in human food. The empirical formula of 7-dehydrocholesterol is $C_{27}H_{44}O$, while ergosterol has the formula $C_{28}H_{44}O$. These two provitamins D have the following constitutions:



7-Dehydrocholesterol



Ergosterol

It has already been stated that provitamins D do not act as vitamins D. They do not have any physiological activity of their own, as far as is known.

All provitamins D are well-crystallized, colorless and odorless compounds. Pure 7-dehydrocholesterol melts at 150–151° C., while ergosterol containing 1.5 moles of water of crystallization melts at 168° C. The melting point of ergosterol varies with the degree of hydration. Complete dehydration is difficult to achieve and results in a product with a melting range from 166 to 183° C. Both provitamins D are optically active: 7-dehydrocholesterol, $[\alpha]_D^{20} = -113.6°$ in chloroform; ergosterol, $[\alpha]_D^{20} = -135°$ in chloroform.

In addition to these provitamins D, the following have been obtained by laboratory methods: epi-7-dehydrocholesterol, epi-ergosterol, 22-dihydroergosterol, 22-oxido-ergosterol, 7-dehydrositosterol, 7-dehydrostigmasterol and 7-dehydrocampesterol. Some of these may also occur in nature. Furthermore, it has been stated that different and new provitamins D occur in mussels and in periwinkles.

All provitamins D have similar solubility characteristics. They are insoluble in water, but soluble in the typical organic solvents such as ethers, hydrocarbons, and fats. The lower alcohols are usually used for recrystallization.

All provitamins have the same characteristic absorption spectrum in the ultraviolet which is characterized by maxima at 260, 270, 281, and 293.5 mμ and on which a quantitative method for the determination of the provitamins is based.

The provitamins D are generally relatively stable compounds, but are sensitive to light.

Chemically, the provitamins D are simple derivatives of sterols (see page 99) and differ from them only by the presence of one additional double bond. While all sterols have a double bond in the 5,6-position, the provitamins D have another one in conjugation, namely, in 7,8-position of the sterol molecule.

(*b*) *Conversion of Provitamins D to Vitamins D*

The conversion of provitamins D to vitamins D cannot be accomplished by the organism without outside help. This consists in the ultraviolet radiation of the sun. In order to derive as much activation as possible from sunlight, the provitamin D is concentrated in the skin of animals. Feather- and fur-bearing animals have an unusually high concentration of provitamin in the uncovered part of the body; for example, chickens have a high concentration of provitamin D in their feet.[21]

The conversion of provitamins D to vitamins D is a process which can also be carried out in the laboratory and which is used in the commercial production of vitamin D. Ultraviolet light is usually used for this process, but other activation procedures are sometimes used, such as electrons of high frequency.

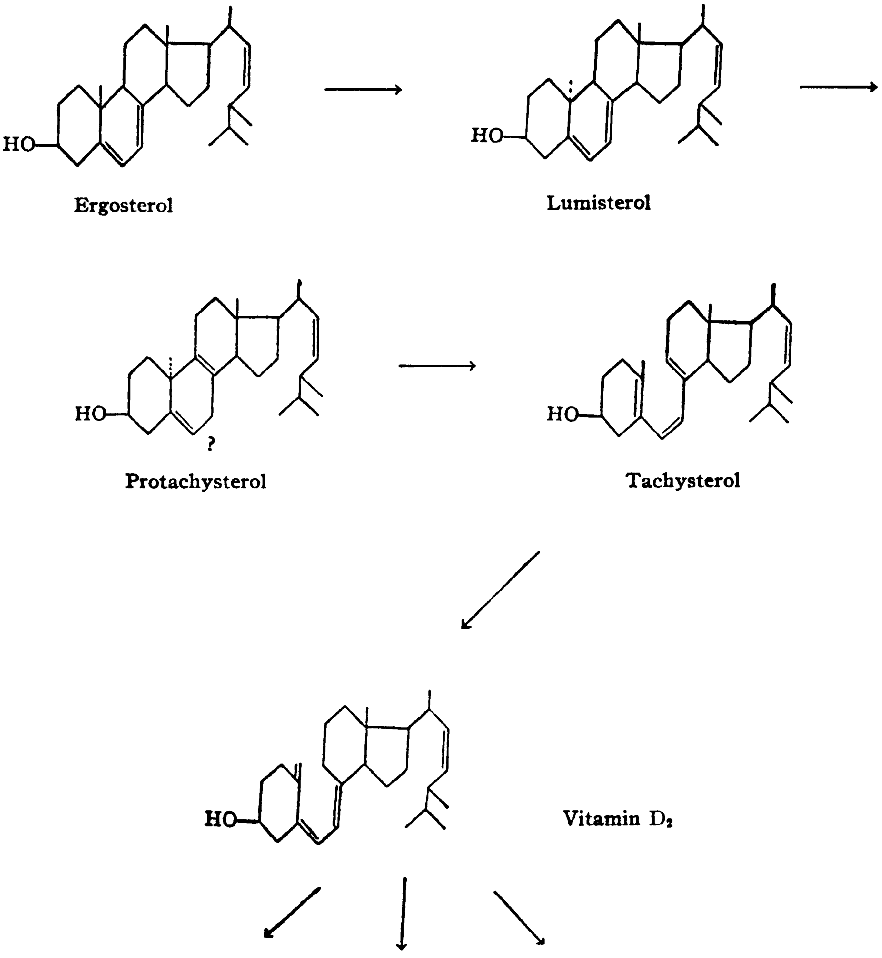
The activation is a complicated process. Vitamin D is not formed directly from the provitamin. There are two or probably three intermediates which are produced consecutively before vitamin D is obtained. Vitamin D, on the other hand, is not stable to the activating energy but is converted by it into a series of other compounds. None of the irradiation products, other than the vitamin itself, has vitamin D activity. The mechanism of this conversion is illustrated by the series of formulas on page 248, in which ergosterol is used as an example and in which the sterol nucleus is indicated in an abbreviated manner.

(*c*) *Vitamins D*

The number of naturally occurring vitamins D is unknown. According to an analysis by means of the molecular distillation technique six different vitamins D are present in cod-liver oil.[22] Only two vitamins D have been isolated from fish oils in pure form, namely, the vitamins derived from 7-dehydrocholesterol and ergosterol. These compounds are called vitamin D_3 and vitamin D_2, respectively. The latter is also called *viosterol* in America and *calciferol* in Europe. In addition to these vitamins D there are the vitamins obtained from the various provitamins D, which have been previously enumerated. (There is no vitamin D_1. This term had erroneously been assigned to a molecular addition product of vitamin D_2 with lumisterol.)

---

[22] K. C. D. Hickman and E. L. Gray, *Ind. Eng. Chem.*, 30, 796 (1938).



Ergosterol

Lumisterol

Protachysterol

Tachysterol

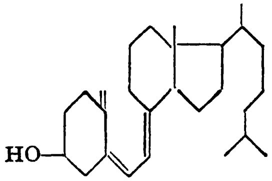
Vitamin D_2

Toxisterol Suprasterol I Suprasterol II

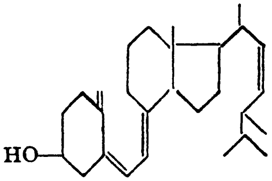
From a practical point of view the vitamins D are differentiated according to the source, namely, animal type and plant type vitamin D. Vitamin D_3 or activated 7-dehydrocholesterol is the representative of the animal type vitamin D, while vitamin D_2 or activated ergosterol represents the plant type vitamin D. This differentiation is necessary since in the nutrition of poultry only the animal type vitamin D is of practical value.

Accordingly, two different standards for vitamin D must be recognized. The "A. O. A. C. chick unit," devised by the Association of Official Agricultural Chemists, is a measure of the animal type vitamin D and is based on the biological response of the U. S. Pharmacopoeia Reference Cod Liver Oil. The I. U. and U. S. P. Unit of vitamin D is a measure of the plant type vitamin D. Vitamin D_3 has a biological efficacy of 40,000,000 A. O. A. C. chick units, I. U. or U. S. P. units per gram. Vitamin D_2 has an efficacy of 40,000,000 I. U. or U. S. P. units per gram. One I. U. is defined as 0.025 γ of the pure vitamin D_2.

The chemistry of the vitamins D is obvious from the discussion of the chemistry of the provitamins D and the reactions involved in the activation mechanism. The various vitamins D differ from each other only in the side chain attached to the opened sterol nucleus. All vitamins D are isomers of the corresponding provitamins. The formulas of vitamin D_3 and vitamin D_2 are as follows:



Vitamin D_3
Activated 7-dehydrocholesterol
M. p. 82–83° C.
$[\alpha]_D^{20} = +83.5$ in acetone



Vitamin D_2
Activated ergosterol
M. p. 115–117° C.
$[\alpha]_D^{20} = +82.6°$ in acetone

All the known vitamins D, in the pure state, are white, odorless crystals. They are soluble in fats and in the usual organic solvents, for example, in ether, chloroform, acetone, alcohol, and are insoluble in water. They exhibit a characteristic absorption spectrum with one maximum at 265 mμ.

The vitamins D are relatively stable against oxidation when pure, but oxidize easily in the presence of the other irradiation products of the provitamins D. They are stabilized, however, in oil solution. They are destroyed by light and by excessive heat.

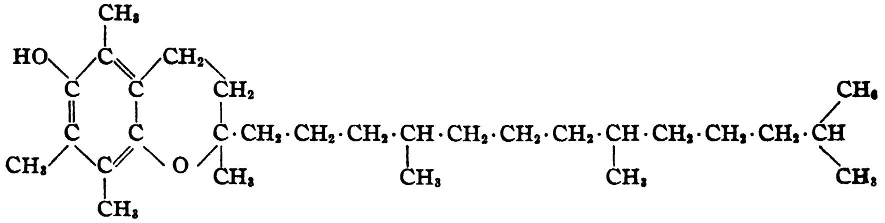
Vitamins D occur in small quantities in most animals. Significant amounts are found only in eggs, in milk, and in liver. The liver of many fish contains abundant quantities.

Vitamin D is needed especially by babies and young children. The optimum daily dose is about 400 to 800 units. For man the animal type vitamin D is about 1.5 times more efficient than the plant type vitamin D.

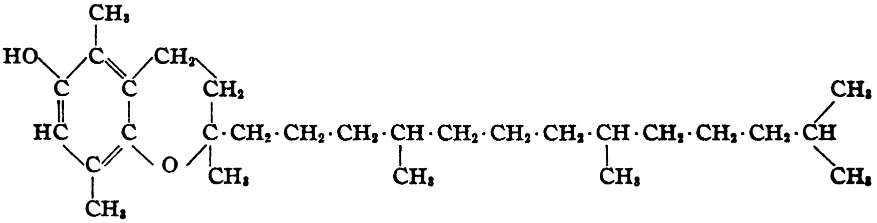
A deficiency in vitamin D causes the occurrence of *rickets* which is characterized by insufficient deposition of calcium and phosphorus in the growing bones. This results in typical skeletal changes which are noticeable in the ribs, forearms, wrists, and legs. In babies the retardation of the calcification of the fontanelles is especially characteristic. Instead of the calcification of the bones, excessive cartilage formation sets in especially at the ends of the long bones and results also in enlargements of the junctions between the bones. Deficiency of vitamin D is to a certain extent but not altogether responsible also for the occurrence of dental caries. Deficiency of vitamin D in children may also be the cause of infantile *tetany* which is usually the sign of the beginning of healing, and occurs when the faulty phosphorus metabolism is relieved while the calcium metabolism is still impaired. Rickets of the adult is known as *osteomalacia* and is characterized by a decalcification of the bones which leads to brittleness.

11. The Group of Vitamins E[23]

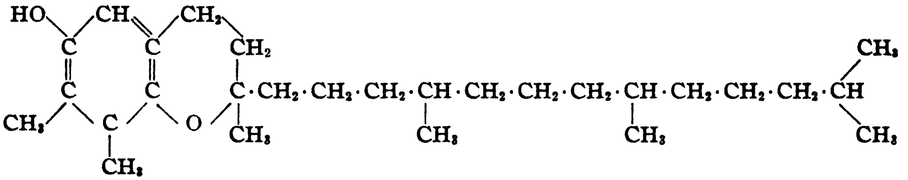
Three different vitamins E have been isolated from natural sources and it appears possible that additional compounds with the same physiological effect occur naturally. The vitamins E are known as *α-tocopherol,* $C_{29}H_{50}O_2$, *β-tocopherol,* $C_{28}H_{48}O_2$, and *γ-tocopherol,* $C_{28}H_{48}O_2$. They have the following constitutions:



α-Tocopherol



β-Tocopherol



γ-Tocopherol

The biological activity of the vitamins E is measured according to the I. U. by the activity of the synthetic racemic α-tocopherol acetate (*dl*-α-tocopherol acetate), 1 g. of which contains 1000 I. U. Therefore, one I. U. corresponds to 1 mg. of the standard. The synthetic racemic α-tocopherol has the same biological efficacy as the naturally occurring α-tocopherol. The β- and γ-isomers are only half as active as the α-isomer.

The tocopherols are oils which have not been obtained in the crystalline state. Certain esters, however, such as the allophanates and 3,5-dinitrobenzoates are crystalline.

---

[23] L. I. Smith, *Chem. Revs.*, **27**, 287 (1940). Society of Chemical Industry, *Vitamin E.* Chemical Pub. Co., New York, 1940.

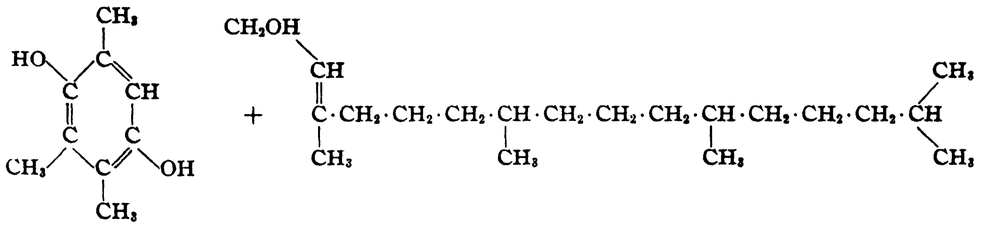
The tocopherols have a characteristic absorption spectrum in the ultraviolet with a maximum at 295 mμ which is displaced to 285 mμ in the esters such as the acetates.

In the absence of oxygen, the vitamins E are stable to heat treatment up to 200° C. and are not affected by mineral acids up to 100° C. Alkali destroys the vitamins of this group only very slowly, so that they can be obtained by alkaline saponification. The tocopherols are, however, sensitive to oxidation which destroys their biological activity.

The vitamins E are soluble in all lipoid solvents, but are insoluble in water. They are fairly stable to visible light, but are destroyed readily by ultraviolet light.

The tocopherols are potent antioxidants, the γ-isomer being more effective than the β-isomer, which in turn is more effective than the α-isomer. Thus the antioxidant power is approximately the reverse of the vitamin activity and is dependent upon the presence of a free phenolic hydroxyl group which is not necessarily important for the vitamin action.

Chemically, the tocopherols are chroman derivatives. While the α-isomer contains three methyl groups attached to the benzene ring the β- and γ-isomers have only two methyl groups, in the *p*- and *o*-position, respectively. All three have a phenolic hydroxyl group in *p*-position to the ring-oxygen, and all have the same characteristic terpinoid side chain attached to the carbon atom next to the ring-oxygen. The chemical structure of the tocopherols and of the side chain specifically can be described best by indicating a method of synthesis according to which an alkylated hydroquinone is condensed with phytol:



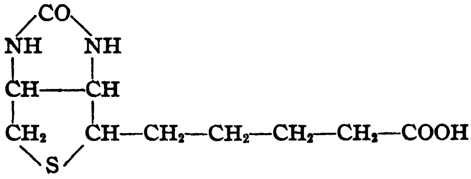
The tocopherols occur predominantly in plant materials. The animal organism contains only small amounts. Vegetable oils are the best source, but lettuce and beans also contain considerable amounts. Usually a mixture of at least two of the tocopherols is present and the relative amounts vary considerably. The vitamins E are present in plant oils as the free compounds, but small amounts of esters may also occur.

It is believed, but not definitely proved, that vitamin E is necessary for the human organism. The daily dose may be of the order of 10 mg. The mechanism of the vitamin E action is obscure but may be due to the ability of the molecule to transfer hydrogen.

Vitamin E deficiency in rats causes sterility in both the male and female animal. The information as to the effects of vitamin E on the human organism is scanty and must be regarded as provisional. Considerable evidence indicates that vitamin E may exert beneficial influence in certain cases of habitual abortion and possibly also on threatened abortion and premature separation of the placenta.

12. Vitamin H—Biotin[24]

Vitamin H is more generally called *biotin* and can also be classified as a member of the vitamin B complex. Biotin has the empirical formula $C_{10}H_{16}O_3N_2S$ and has the following chemical structure:



Biotin has the biological activity of 27,000,000 vitamin H units per gram. One vitamin H unit is 0.04 γ.

Biotin crystallizes in long, thin needles, melting at 230–232° C. It is water- and alcohol-soluble but is relatively insoluble in chloroform, ether, and petroleum ether. Biotin is optically active, $[\alpha]_{D}^{22} + 92°$ in 0.1 *N* sodium hydroxide solution.

Biotin is resistant to treatment with acids or alkalies at ordinary temperatures and is also stable against air and light. Higher temperatures or treatment with oxidizing agents destroy the vitamin.

Chemically, biotin is a bicyclic compound of acid character. One of the rings has the constitution of tetrahydrothiophene while the other one is also a five-membered ring containing two nitrogens in urea configuration. A valeric acid side chain is attached to one of the carbon atoms adjacent to the ring-sulfur.

Biotin occurs in very small amounts in all higher animals and appears to occur in higher concentrations in plants. It occurs predominantly in the free state in fruits and in grasses. In grains, nuts, and vegetables this vitamin is present partly in a bound form and partly free. In yeast and in animal tissues, for example in liver, biotin occurs mainly as a chemically bound compound.

Biotin is believed to play an essential role in all animals and in man. The requirements have, however, not been established and the mechanism of the vitamin action is also unknown.

Biotin deficiency in man has not been thoroughly studied. Experimental biotin deficiency on human subjects[25] resulted in the occurrence of a number of clinical symptoms similar to those of spontaneous avitaminosis. Extreme lassitude and somnolence, muscle pains, precordial distress, and anorexia were observed. There appeared also a striking, ashy pallor of the skin and the mucous membranes followed by an increasing dryness of the skin. Rats and chicks develop peculiar and impressive skin changes on when fed a diet deficient in biotin.

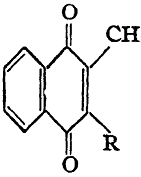
---

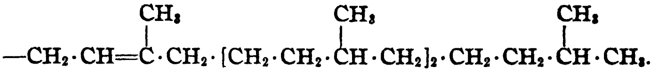
[24] V. du Vigneaud, *Science*, **96**, 455 (1942).

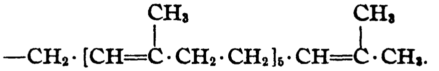
[25] V. P. Sydenstricker, S. A. Singal, A. P. Briggs, N. M. DeVaugh, and H. Isbell, *Science*, **95**, 176 (1942).

13. The Group of Vitamins K[26]

Two different compounds with vitamin K activity have been isolated from natural sources. They are called vitamin K_1 and vitamin K_2. It is, however, conceivable that additional vitamins K occur naturally. Besides these materials a great number of simple chemical compounds have been found to possess vitamin K activity. Of these, one is of outstanding importance since it is widely used in human and animal nutrition as a cheap substitute. This compound is 2-methyl-1,4-naphthoquinone. The vitamins K_1 and K_2 are derivatives of the latter and are characterized by the presence in the 3-position of a β-unsaturated side chain of isoprene structure. Vitamin K_1 has the empirical formula $C_{31}H_{46}O_2$ and that of vitamin K_2 is $C_{41}H_{56}O_2$. They have the following chemical structures:



Vitamin K_1: R =

$$—CH_2·CH{=}\overset{CH_3}{\overset{|}{C}}·CH_2·[CH_2·CH_2·\overset{CH_3}{\overset{|}{C}}H·CH_2]_2·CH_2·CH_2·\overset{CH_3}{\overset{|}{C}}H·CH_3.$$

Vitamin K_2: R =

$$—CH_2·[CH{=}\overset{CH_3}{\overset{|}{C}}·CH_2·CH_2]_5·CH{=}\overset{CH_3}{\overset{|}{C}}·CH_3.$$

2-Methyl-1,4-naphthoquinone: R = H.

The biological activity of the vitamins K and their substitutes has been expressed by a variety of different units. The compounds are used, however, on the weight basis rather than by reference to biological units.

Vitamin K_1 is a yellow oil with a melting point of about −20° C. Vitamin K_2 is a yellow crystalline compound, m. p. 53.5–54.5° C. 2-Methyl-1,4-naphthoquinone is a lemon-yellow crystalline powder with a faint but characteristic odor, m. p. 106° C. Vitamins K_1 and K_2, but not the 2-methyl-1,4-naphthoquinone, exhibit a white fluorescence in the light of an argon lamp. The vitamins K exhibit typical absorption characteristics in the ultraviolet region with maxima at 243, 248, 261, 270, and 328 mμ. The 2-methyl-1,4-naphthoquinone shows bands only at 243 and 328 mμ.

The vitamins K are soluble in most of the common organic solvents, especially in ether, hexane, acetone, etc., but are insoluble in water and only sparingly soluble in methanol. 2-Methyl-1,4-naphthoquinone, however, is soluble in water to the extent of 0.13 mg. per ml. The vitamins K are essentially thermostable. They are very sensitive to alkali and to light from various sources.

Vitamin K_1 occurs predominantly in plants, especially in the green leafy tissues.

---

[26] E. A. Doisy, S. B. Binkley, and S. A. Thayer, *Chem. Revs.*, **28**, 477 (1941). H. Dam, in *Advances in Enzymology*, Vol. II. Interscience, New York, 1942, p. 286. H. R. Butt and A. M. Snell, *Vitamin K.* Saunders, Philadelphia, 1941.

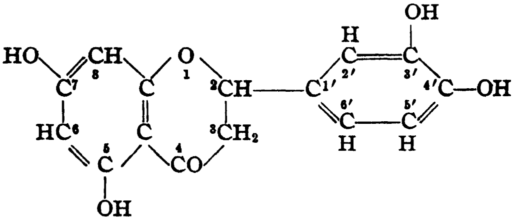
Vitamin K_3 is produced mainly by microorganisms, especially by bacteria including the species which thrive in the intestinal tract of many higher animals.

Vitamin K is essential for man, and is usually given in doses ranging from 1 to 10 mg. The mechanism of vitamin K action is unknown, but it has been suggested that the quinone structure of the vitamin takes part in an oxidation-reduction system.

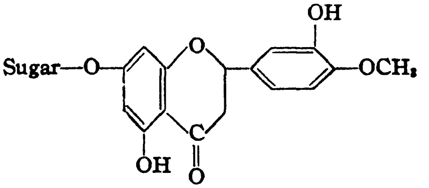
Vitamin K deficiency is characterized mainly by the occurrence of hemorrhages. These are caused by a marked decrease of the prothrombin content of the blood which results in a considerable prolongation of the blood-clotting time.

14. Vitamin P[27]

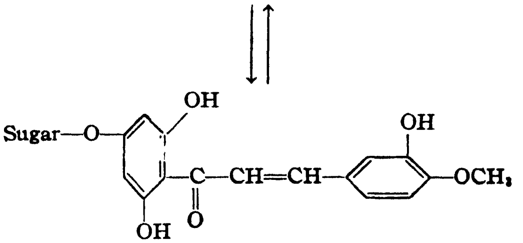
Vitamin P is also called *citrin* and appears to be a mixture of two glucosides, hesperidin and eriodictin. They yield upon hydrolysis hesperitin and eriodictyol. These are flavanone derivatives, hesperitin being the 4′-methyl ether of eriodictyol. The latter has the following chemical constitution:



The flavanone is apparently in equilibrium with the open chalcone isomer:



Hesperidin



Hesperidin chalcone

The chalcone is apparently the naturally occurring compound and has been isolated as such. The chalcone of hesperidin forms bright yellow microscopic crystals, m. p.

---

[27] C. Z. Wawra and J. L. Webb, *Science*, **96**, 302 (1942).

251–252° C. while hesperidin is a colorless compound, m. p. 261–262° C. The chalcone is soluble in pyridine, very slightly soluble in methanol, and insoluble in water. It shows a characteristic ultraviolet absorption with maxima at 332 and 308 $m\mu$.

Vitamin P occurs within tissues in combination with a protein. The mechanism of the vitamin P action is believed to be a function of the demonstrated ability of this protein complex to serve as a hydrogen transporter.

The exact vitamin P requirement of man is not known. In clinical cases 50–150 mg. of the flavanone mixture have been administered.

In patients suffering from vitamin P deficiency, capillary resistance is decreased and vascular permeability is increased. The clinical symptoms comprise scurvy-like hemorrhagic conditions of the skin which cannot be cured by vitamin C administration.

15. Nonidentified Vitamins

In addition to the vitamins which have been identified there is an unknown number of nonidentified vitamins. A considerable list of suggested new dietary essentials follows, but it remains to be seen how many of these will prove to be separate entities. On the other hand, there is the definite possibility that future research will uncover other vitamins, the existence of which is unknown today.

(*a*) *Vitamin* B_3, as a member of the vitamin B complex, is said to be soluble in water and dilute alcohol and to be sensitive to heat and alkali. It has been suggested that vitamin B_3 is identical with pantothenic acid.

(*b*) *Vitamin* B_4 may be identical with a mixture of arginine and glycine.

(*c*) *Vitamin* B_5 is probably identical with nicotinamide.

(*d*) *Vitamin* B_7 is also called "vitamin I."

(*e*) *Vitamin* B_8 is of purine character and is probably adenylic acid or adenine.

(*f*) *Vitamin* B_c.

(*g*) *Vitamin* B_p.

(*h*) *Vitamin J* has also been referred to as vitamin C_2.

(*i*) *Vitamins* L_1 *and* L_2 are claimed to be water-soluble compounds.

(*k*) *Vitamin M.*

(*l*) *Factor U.*

(*m*) *Folic Acid.*

(*n*) *Growth Factor for Lactobacillus casei.*

(*o*) *Grass Juice Factor.*

(*p*) *Mouse Factor.*

(*q*) *Guinea Pig Factors 1, 2, and 3.*

(*r*) *Anti-Pernicious Anemia Factor.*

II. THE VITAGENS[28]

There are five chemically different groups of compounds which on the basis of their particular function within the animal organism are classified[28] as vitagens. These comprise the essential fatty acids; the essential amino acids; the essential carbohydrates; choline and related compounds and the essential transferable methyl group; and finally the essential organic sulfur-containing compounds.

---

[28] H. R. Rosenberg, *Chemistry and Physiology of the Vitamins.* Interscience, New York, 1942.

1. Essential Fatty Acids[29]

Each of the following three fatty acids is known to act as an essential fatty acid: linoleic acid, linolenic acid, and arachidonic acid. These compounds have the following structural formulas:

$$CH_3\cdot(CH_2)_4\cdot CH{=}CH\cdot CH_2\cdot CH{=}CH\cdot(CH_2)_7\cdot COOH$$

Linoleic acid

$$CH_3\cdot CH_2\cdot CH{=}CH\cdot CH_2\cdot CH{=}CH\cdot CH_2\cdot CH{=}CH\cdot(CH_2)_7\cdot COOH$$

Linolenic acid

$$CH_3\cdot CH_2\cdot CH_2\cdot CH_2\cdot(CH_2\cdot CH{=}CH)_4\cdot CH_2\cdot CH_2\cdot CH_2\cdot COOH$$

Arachidonic acid

There are a number of other highly unsaturated fatty acids which occur naturally but their vitagen nature has not been investigated as yet.

Linoleic acid is a colorless liquid at room temperature, m. p. 11° C. Linolenic acid is characterized by its b. p. 230–232° C./17 mm. Hg. Arachidonic acid forms colorless crystals, m. p. 77° C. All essential fatty acids have similar solubility characteristics. They are insoluble in water, but soluble in alkali and in organic solvents.

Linoleic and linolenic acids occur in vegetable oils. Arachidonic acid occurs predominantly in animal tissues.

The requirements of man for the essential unsaturated fatty acids have not been quantitatively investigated. Arachidonic acid appears to be somewhat more effective than linoleic acid, while linolenic acid is at best one-third as active as linoleic acid.

There are certain observations which indicate that a deficiency of the essential fatty acids causes the occurrence of certain eczema and especially infantile eczema. In rats a deficiency of these vitagens causes a typical dermatosis characterized by a general dryness of the skin and thinness with much scurf, especially on the paws, ears, and tail.

2. Essential Amino Acids[30]

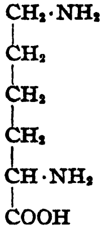
Among the amino acids which are normal constituents of dietary proteins, ten are essential. In the past, "essential amino acids" were defined as those amino acids which must be present in the diet of young rats, in addition to all other energy and building unit requisites and the vitamins, to insure optimal growth. In the future, interest will be focused on the amino acids which are indispensable to man. Amino acids which the organism is either unable to synthesize or at least cannot synthesize at the rate necessary for normal growth are termed essential amino acids.

---

[29] G. O. Burr, *Federation Proceedings*, **1**, 224 (1942).

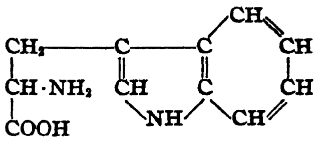
[30] W. C. Rose, *Physiol. Revs.*, **18**, 109 (1938); *Science*, **86**, 298 (1937).

The ten essential amino acids are: lysine, tryptophane, histidine, phenylalanine, leucine, isoleucine, threonine, methionine, valine, and arginine.

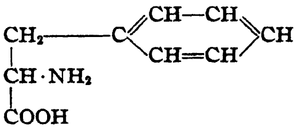


$CH_2\cdot NH_2-CH_2-CH_2-CH_2-CH\cdot NH_2-COOH$

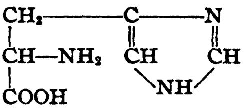
Lysine
(α,ε-Diaminocaproic acid)



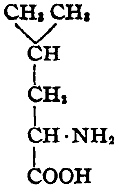
Tryptophane
(β-Indolylalanine)



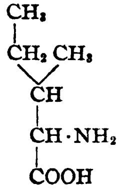
Phenylalanine



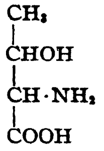
Histidine
(β-Iminazolylalanine)



Leucine
(α-Aminocaproic acid)

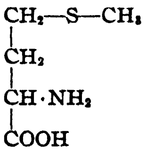


Isoleucine
(α-Amino-β-methylvaleric acid)



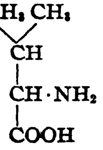
$CH_3-CHOH-CH\cdot NH_2-COOH$

Threonine
α-Amino-β-hydroxybutyric acid)

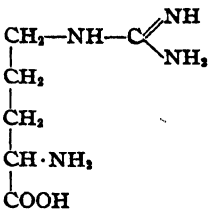


$CH_3-S-CH_2-CH_2-CH\cdot NH_2-COOH$

Methionine



Valine
(α-Aminoisovaleric acid)



Arginine

These amino acids are all colorless crystalline compounds. They are all relatively insoluble in alcohol and relatively soluble in water. They are optically active. See Table 28.

The exact requirements of the essential amino acids for man are not known. Tryptophane, methionine, valine, threonine, leucine, isoleucine, phenylalanine, and lysine have been proved to be dietary essentials for the human organism on the basis of nitrogen balance studies. Histidine apparently is not required in the diet of man for nitrogen equilibrium, nor is arginine, but the latter is essential for normal spermatogenesis.

The minimum amount of the vitagens necessary to support normal growth has been determined only for rats. The total amount of essential amino acids corresponds to 5.8% of the food consumed with the following figures for the individual amino acids: lysine 1.0%, leucine 0.9%, phenylalanine 0.7%, valine 0.7%, threonine 0.6%, methionine 0.6%, isoleucine 0.5%, histidine 0.4%, tryptophane 0.2%, and arginine 0.2%. It is known that the same figures do not necessarily apply for other animals as has been shown with dogs and chicks.[31]

TABLE 28

SOLUBILITY AND OPTICAL ACTIVITY OF THE ESSENTIAL AMINO ACIDS

	m. p., ° C.	$[\alpha]_D$	Solubility in H_2O
l(+)-Lysine	224	+15.3°	Soluble
l(−)-Tryptophane	289	−34°	Soluble, hot only
l(−)-Histidine	277	−39.7°	Soluble
l(−)-Phenylalanine	278	−35.3°	1:32.4 (25°)
l(−)-Leucine	337	−10.4°	1:45 (20°)
l(+)-Isoleucine	280	+ 9.7°	1:25.8 (15.5°)
l(−)-Threonine	257	−27.7°	Soluble
l(−)-Methionine	283	− 6.87°	Soluble
l(+)-Valine	315	+ 6.4°	Soluble
l(+)-Arginine	238	+26.5°	Soluble

A deficiency of any one or all of the ten essential amino acids causes an inhibitory effect upon growth. There are some specific deficiency symptoms known to occur in animals. Thus, the fright disease of dogs has been correlated with an inadequate supply of amino acids. A deficiency of lysine causes anemia in rats. Cataract and other ocular changes have been observed in rats on a tryptophane-deficient diet. Deprivation of valine results in a disorder of the nervous system. Chicks on a diet devoid of arginine and glycine develop a typical paralysis. Specific deficiency symptoms of man are being studied.

3. Essential Carbohydrates[32]

It has been shown that some fats and proteins function as vitagens. In a similar manner certain specific carbohydrates may be vitagens. Ascorbic acid and inositol are examples of essential carbohydrates which are vitamins. Whether or not other carbohydrates exist which are essential food components is not known. If carbohydrates as food components are utilized only to supply energy, it should be possible to replace all carbohydrates with the exception of the carbohydrates of vitamin character by some other energy-bearing foods such as fats or proteins. This is, however, not the case. The animal organism maintains, for example, in the blood a certain normal glucose concentration and it has been shown that the organism is able to synthesize a certain amount of glucose for this purpose. Animals on a completely carbohydrate-free diet, however, do not survive, apparently because the amount of glucose synthesized is not sufficient to maintain the necessary carbohydrate concentration in the tissues and body fluids. It is thus possible that either carbohydrates in general or some specific carbohydrates may eventually be found to be essential protective food components.

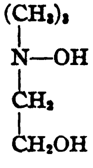
---

[31] H. J. Almquist, *Federation Proceedings*, 1, 269 (1942).

[32] H. R. Rosenberg, *Chemistry and Physiology of the Vitamins*. Interscience, New York, 1942.

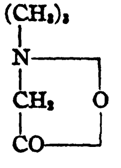
4. Choline and Related Compounds and the Essential, Transferable Methyl Group[33]

The number of naturally occurring compounds which may serve as suppliers of the essential transferable methyl group is not known. Choline appears to be the most important compound, but other materials such as methionine and betaine are known to act in this capacity, especially in the absence of the former. Methionine has already been mentioned among the essential amino acids. On the other hand, there are some observations which are interpreted as indicating that choline may possess, in addition to its activity as a supplier of transferable methyl groups, some other function, the mechanism of which is distinctly different from that of methyl transfer.

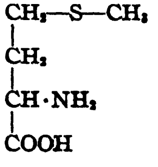


$$\begin{array}{l}(CH_3)_3\\ |\\ N{-}OH\\ |\\ CH_2\\ |\\ CH_2OH\end{array}$$

Choline



Betaine



$$\begin{array}{l}CH_3{-}S{-}CH_2\\ |\\ CH_2\\ |\\ CH{\cdot}NH_2\\ |\\ COOH\end{array}$$

Methionine

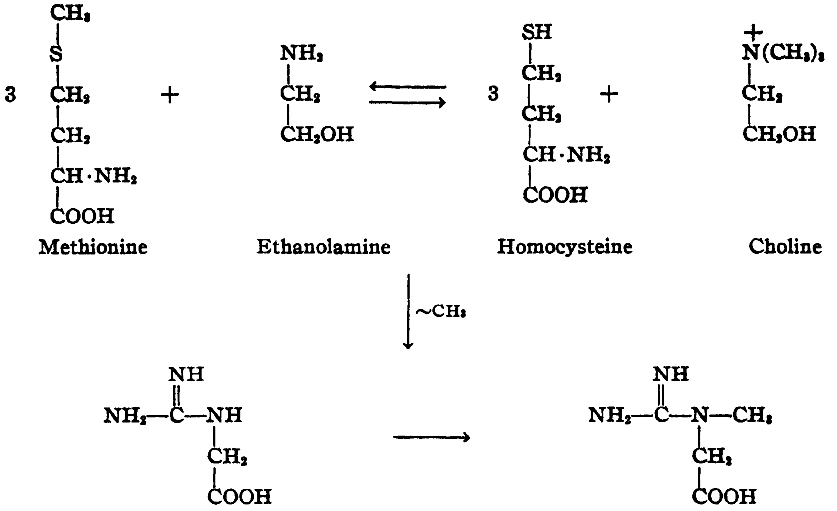
Choline is a colorless, viscid, strongly alkaline liquid and is very hygroscopic. It absorbs carbon dioxide from the air and forms salts easily, for example the chloride, borate, picrate, etc. Choline is very soluble in water and alcohol, but is insoluble in ether. The salts are white, hygroscopic crystals which are soluble in water and in alcohol. Their aqueous solutions are practically neutral.

Betaine forms very hygroscopic crystals which lose water at 100° C. It melts at 293° C. under rearrangement into the methyl ester of dimethylaminoacetic acid.

Some of the anabolic reactions involving the transfer of the methyl group are shown in the following scheme:

---

[33] E. W. McHenry, W. H. Griffith, D. Glick, and V. du Vigneaud, "Symposium on the Biochemistry of Choline," *Biol. Symposia*, 5, 177 (1941). W. H. Griffith, *J. Nutrition*, 22, 239 (1941).



Methionine Ethanolamine Homocysteine Choline

Guanidinoacetic acid Creatine

The choline requirements of the human organism are not known. Dogs need about 35 mg. per kg. of body weight. In the absence of choline, the animal organism is able to utilize methionine or betaine in an estimated efficacy of about 30%.

A deficiency of choline in man is not known. In rats a paralysis of the hind legs occurs. In chicks choline deficiency causes decreased egg production, increased mortality, and abortion of egg yolks. In poultry a deficiency of this vitagen is characterized by perosis which results in a bending and twisting effect of the bones.

5. Essential Organic Sulfur-Containing Compounds[32]

There is the possibility that some organic chemical compounds containing sulfur belong to the class of vitagens. Sulfur-containing organic compounds act not only as structural building units, for example in hair and nails, but are also of specific and functional importance for growth and maintenance of life. A deficiency of sulfhydryl groups brings about death. Sulfhydryl compounds apparently act as activators and inhibitors of a number of enzyme systems, probably by virtue of their ability to undergo reversible oxidation to disulfide compounds and reduction to the original sulfhydryl compounds.

III. THE HORMONES[34]

Most reactions occurring in the organism are regulated by special chemical compounds, known as hormones. These hormones act in small concentrations and in a manner the mechanism of which is unknown. It is believed that every specific action within the organism and within the cell is governed by a specific hormone, but this is extremely difficult to prove in all cases, such as in the case of the *organizer* in the egg cells. Among the compounds which are definitely regarded as hormones are the substances which determine growth and reproduction, which assure constant heart beat and constant blood pressure, and substances which regulate anabolism and catabolism. There are other hormones, such as the so-called wound hormones and neurohormones. Different hormones occur in lower animals such as a pupation hormone and a sex-determining hormone.

---

[34] *"Glandular Physiology and Therapy,"* Am. Med. Assoc., Chicago, 1942.

A few of the many postulated hormones have been isolated and are chemically known. Only these will be discussed.

The term hormone, as it is commonly used, applies only to those hormones which are believed to originate in the ductless glands. These compounds, which are also called endocrine hormones, comprise the great majority of the chemically known hormones.

1. The Pituitary Hormones[34]

The pituitary gland produces in its three anatomically different parts, the posterior lobe, the anterior lobe, and the pars intermedia, a number of different hormones. They appear to consist of protein material, none of which have been isolated in the pure state. As a result, the occurrence of different hormones has been suggested solely on the basis of their apparent function. The following hormones are, however, believed to be separate compounds.

The posterior lobe produces hormones with the ability to produce uterine contraction which is called *α-hypophamine*, *oxytocin* or *pitocin* and a second hormone with the ability to raise blood pressure which is called *β-hypophamine*, *vasopressin* or *pitressin*. The latter compound is probably also responsible for the power of extracts of the posterior lobe to produce diuresis and anti-diuresis, respectively.

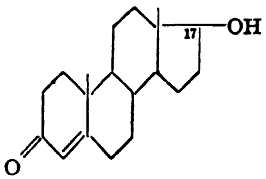
The pars intermedia produces a hormone which causes a dispersion of the black pigment granules of frogs and other amphibia. This hormone is called *intermedin* and appears to be involved in the control of the metabolism in higher animals. It decreases. carbohydrate consumption and increases combustion of fat.

The anterior lobe produces at least six different hormones. There is first the *growth principle*. Its absence causes dwarfs, while an excessive secretion produces giants. There are two gonadotropic hormones, one of which stimulates male germ cells and ovarian follicles while the other is necessary for the formation of the corpus luteum (luteinizing hormone). The latter hormones are called *prolan A* and *B* and may or may not be separate entities. The follicle-stimulating hormone is probably identical with the gonadotropic hormone which occurs in relatively high concentration in urine during early pregnancy (basis for the Aschheim-Zondek test for pregnancy). In addition, the anterior pituitary produces the *thyreotropic hormone* which stimulates the thyroid and causes secretion of thyroxin. Through the thyroid the thyreotropic hormone influences the basal metabolic rate. The existence of a *parathyreotropic* hormone which governs the parathyroid is also postulated. Lactation depends upon pituitary function and the hormone responsible for this action is known as the *lactogenic hormone* or *prolactin*. This hormone is found also in liver, placenta, milk, and urine. The last of these, the *corticotropic hormone*, stimulates the adrenal cortex and the production of the hormones in these tissues. There may also be a *medullotropic principle* which directs the production of adrenaline, and a *pancreatropic hormone* which directs the production of insulin.[35]

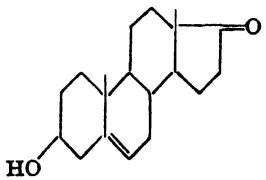
2. The Androgenic Hormones[36]

The male sex hormones are called androgens. They are necessary for the normal development of the male sex organs and characteristics. While a great number of androgenic compounds have been obtained by synthesis, only a few have been isolated from natural sources. The most important of these is *testosterone* which occurs in the testes and which is apparently the only true physiological male sex hormone. Two additional compounds, *androsterone* and *trans-dehydroandrosterone*, have been isolated from urine and are most probably metabolic end products of testosterone. It is interesting to note that an androgenic compound has also been found in the adrenal cortex and is called *adrenosterone*. Of all of these compounds only testosterone has found medical application. It is used mainly as the propionate which is more active than the free hormone. Besides testosterone, *17-methyltestosterone*[37] has become an important androgen for clinical therapy since it can be given orally, while testosterone is practically devoid of androgenic activity when given per os.

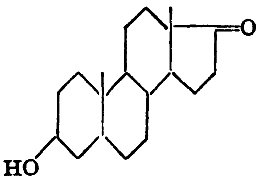
Androgens are sterol derivatives and differ from sterols by the absence of the side chain. They are characterized by the presence of oxygen in the 17-position which carries the side chain in the sterols. All of these compounds have been synthesized by chemical degradation of cholesterol.



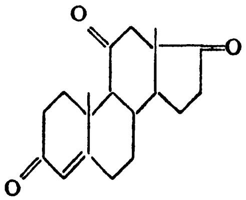
Testosterone



Transdehydroandrosterone



Androsterone



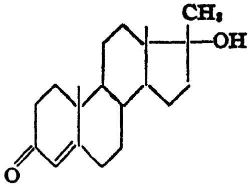
Adrenosterone

---

[35] A symposium concerning the protein hormones of the pituitary body has been published in *Ann. N. Y. Acad. Sci.*, 43, 253 (1943).

[36] F. C. Koch in E. Allen and E. A. Doisy, *Sex and Internal Secretion*. Wood, Baltimore, 1939, p. 807.

[37] L. Ruzicka, M. W. Goldberg, and H. R. Rosenberg, *Helv. Chim. Acta*, 18, 1487 (1935).



17-Methyltestosterone

The amount of testosterone produced in the testes is not known. In clinical therapy doses of 1–5 mg. are usually administered. The effective oral dose of methyltestosterone is 2 to 4 times the effective dose of testosterone administered by injection.

The activity of androsterone, adrenosterone, and trans-dehydroandrosterone is considerably less and is in the order of one-third to one-thirtieth of the testosterone activity. The mechanism of the action of the androgenic hormones in the organism is unknown. The isolation of androsterone and trans-dehydroandrosterone from urine suggests that testosterone may act as a hydrogen transporter in the organism.

3. The Estrogenic Hormones[38]

There are two types of female sex hormones, namely, the follicle hormone and the corpus luteum hormone. The former consists of a group of compounds known as estrogens, while the latter is known as progesterone.

The estrogens govern the growth of the female sex organs and characteristics and are compounds of specific physiological efficacy for the uterus, vagina, and the lactation glands. They are responsible to a certain degree for the maintenance of normal menstruation.

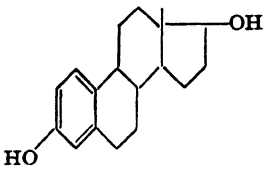
Estrogens have been observed in plant tissues as well as in animal organisms. In mammals, estrogens have been found in the ovary, in the follicle and its fluid, and in the urine. During pregnancy estrogens are formed in the placenta. Considerable amounts are found in the testes and urine of stallions. Compounds with estrogenic activity have also been observed in lower animals such as insects and protozoa.

A number of different estrogens have been isolated from natural sources. The most important compound is *α-estradiol* which differs from the also naturally occurring *β-estradiol* by steric configuration of the nonphenolic hydroxyl group. Estradiol is used clinically, especially in the form of its monobenzoate and is the most active estrogenic compound known. Estradiol has been isolated from ovaries. *Estriol* has been isolated from the placenta and also from pregnancy urine. It is considerably less active than estradiol. In addition *estrone* is obtained from urine. This substance is also known as the α-follicle hormone and contains a keto group in the 17-position. It represents most probably the metabolic end product of the estradiol.

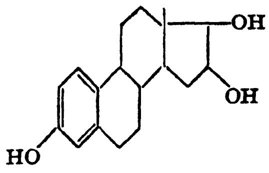
From the urine of pregnant mares three additional compounds of estrogenic character have been isolated which are called *equilin*, *equilenin*, and *hippulin*. These compounds are dehydrogenated estrones and are probably metabolic end products rather than true hormones.

---

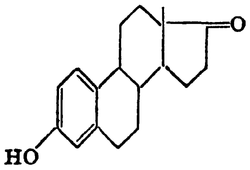
[38] E. A. Doisy in E. Allen and E. A. Doisy, *Sex and Internal Secretion*. Wood, Baltimore, 1939, p. 846.



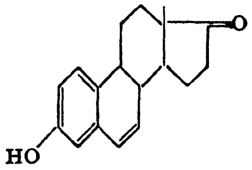
Estradiol



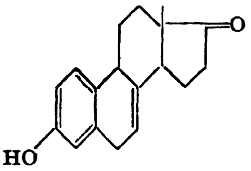
Estriol



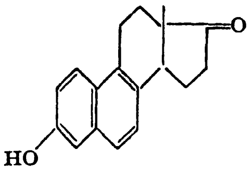
Estrone



Hippulin



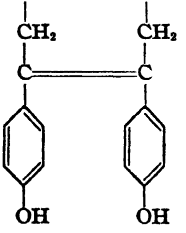
Equilin



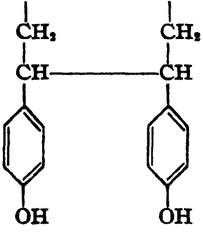
Equilenin

The natural estrogens are sterol derivatives but differ from sterols by the presence of an aromatic ring system and the absence of a methyl side chain which in the sterol molecule is attached to that carbon atom which connects the two rings, one of which has become aromatic in the estrogens. In addition, as is also the case with the androgens, there is no side chain in the 17-position.

None of the estrogenic hormones are available by synthesis. It has been observed, however, that a number of relatively simple chemical compounds exert estrogenic activity. Of these, diethylstilbestrol and hexestrol are most prominent. They are used clinically and are able to replace the difficultly available natural estrogens in all respects. There is the possibility that other synthetic estrogens will be developed.



Diethylstilbestrol



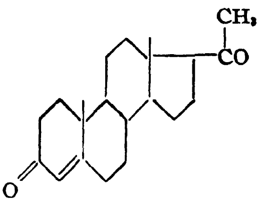
Hexestrol

The amount of estrogens produced within the organism is not known. The quantities found in urine or organs vary considerably as a function of the biological status of the organism. Clinically 0.1–3 mg. of either the natural or the synthetic estrogens are used. The mechanism of the action of these hormones is unknown.

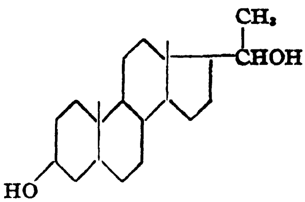
4. The Corpus Luteum Hormone[39]

The corpus luteum hormone is called *progesterone.* This is one of the female sex hormones and the specific action of this substance consists in the preparation of the uterus for pregnancy and in the maintenance of the pregnant state. It is produced in the corpus luteum in the ovary.

Progesterone is a sterol derivative of specific chemical constitution. It is characterized by the presence of a $CH_3 \cdot CO—$ side chain in the 17-position. It is excreted through the urine as pregnanediol which compound does not exhibit progesterone activity.



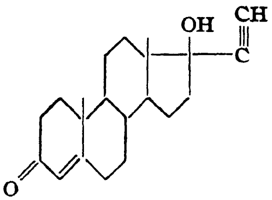
Progesterone



Pregnanediol

Progesterone is obtained synthetically by degradation of the side chain of specific sterols. It occurs in polymorphic forms.

While progesterone appears to be the only naturally occurring compound with the activity of the corpus luteum hormone, there is a synthetic 17-ethinyl compound which is called pregnenin-on-3-ol-17. This compound has one-third of the activity of progesterone when given subcutaneously but is highly active when given per os, while progesterone is relatively inactive when given by this route.



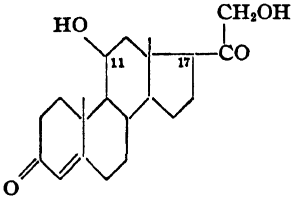
The amount of progesterone produced in the organism is not known. In clinical therapy doses ranging from 1 to 3 mg. are utilized. The mechanism of the hormone action is not known.

---

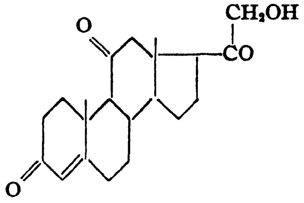
[39] W. M. Allen and E. Allen and E. A. Doisy, *Sex and Internal Secretion.* Wood, Baltimore, 1939, p. 901.

5. The Hormones of the Adrenal Cortex[40]

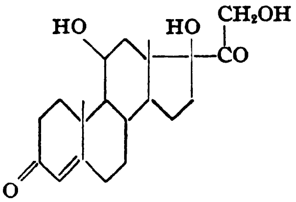
The adrenal cortex produces a number of different compounds with different physiological functions. In total, there are six active compounds which occur in the adrenal cortex. These are *corticosterone, dehydrocorticosterone, 17-hydroxycorticosterone, 17-hydroxydehydrocorticosterone, desoxycorticosterone* and *17-hydroxydesoxycorticosterone.* All these compounds are sterol derivatives and are characterized by a specific side chain, namely, $CH_2OH—CO—$, in the 17-position. They may be classified into two groups, those which contain an oxygen group in the 11-position (corticosterone, dehydrocorticosterone, 17-hydroxycorticosterone and 17-hydroxydehydrocorticosterone), and those which do not contain an oxygenated 11-carbon atom (desoxycorticosterone and 17-hydroxydesoxycorticosterone). The compounds which contain oxygen in the 11-position take part in the regulation of the carbohydrate metabolism, while the compounds of the desoxycorticosterone type are essentially inactive in this capacity. On the other hand, the compounds of the desoxycorticosterone type govern the water and electrolyte metabolism.



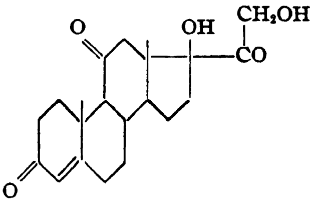
Corticosterone



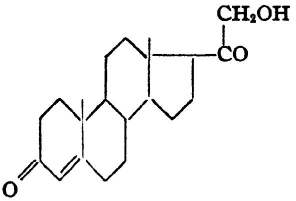
Dehydrocorticosterone



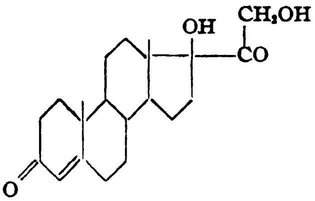
17-Hydroxycorticosterone



17-Hydroxydehydrocorticosterone



Desoxycorticosterone



17-Hydroxydesoxycorticosterone

---

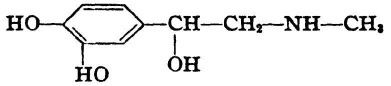
[40] T. Reichstein, *Advances in Vitamins and Hormones.* Vol. 1, Academic Press, 1943. J. J. Pfiffner in *Advances in Enzymology,* Vol. II. Interscience, New York, 1942, p. 325. E. C. Kendall, *J. Am. Med. Assoc.,* **116,** 2394 (1941).

It is not entirely clear as to whether or not all active compounds have been isolated. It is noteworthy, however, that in addition to these six active compounds many inactive materials have been isolated which are chemically very closely related to the active substances but differ from these only in the absence of the double bond and the presence of additional hydroxyl groups. The compounds of the desoxycorticosterone type have been obtained by partial synthesis from sterol compounds.

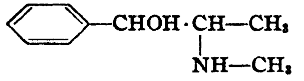
Desoxycorticosterone and corticosterone are used clinically at levels ranging from 1 to 5 mg. They can be administered, in addition to the usual injection method, by implantation of pellets of the crystalline material under the skin.

6. The Hormone of the Adrenal Medulla[41]

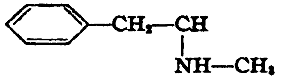
The hormone of the adrenal medulla is known as *adrenaline* or *epinephrin*. It is probably the only active compound of this type occurring in the gland, but this has not been entirely established. Its physiological function consists in stimulating the sympathetic nerve system which becomes evident by an increase in blood pressure. Adrenaline is very active in minute traces and one or two micrograms can be biologically assayed. Chemically, adrenaline is an amine and a phenol. It is available by total synthesis and has the following constitution:



Adrenaline contains an asymmetric carbon atom and is therefore optically active. The natural compound is levorotatory. It is 12 to 15 times more active than the dextrorotatory compound. The material obtained synthetically is the racemic form which, however, can be resolved. Adrenaline is closely related to a number of other physiologically active bases, especially ephedrine and benzedrine, which are more stable than the easily oxidizable adrenaline and which can be given orally while adrenaline must be injected or inhaled.



Ephedrine



Benzedrine

7. The Hormone of the Thyroid[42]

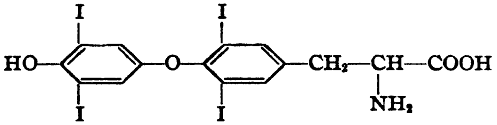
The hormone of the thyroid is called *thyroxine* and governs the basic metabolism. The thyroid gland contains an extremely high concentration of iodine bound in organic iodine compounds. The only compound which has been isolated and which is fully active as a hormone is thyroxine, but it is assumed that additional iodine-containing substances are present and contribute to the activity of the secretions of the thyroid. In addition to thyroxine, *iodogorgoric acid*, which is 3,5-diiodotyrosine, has been isolated from the gland. This acid is, however, only one-five-hundredth as active as thyroxine.

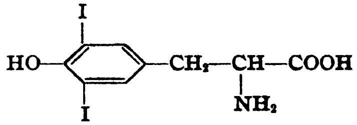
---

[41] C. F. Cori and A. de M. Welch, *J. Am. Med. Assoc.*, **116**, 2590 (1941).

[42] J. Lerman, *J. Am. Med. Assoc.*, **117**, 349 (1941). E. C. Kendall, *Thyroxine*. Chemical Catalog Co., New York, 1929.

Thyroxine is chemically an iodine containing aromatic compound and an aliphatic amino acid. It contains an asymmetric carbon atom and, hence, is optically active. The naturally occurring form is levorotatory. Thyroxine has been obtained by synthesis as a racemic mixture. The dextrorotatory form is approximately one-third as active as the levorotatory.


Thyroxine


Iodogorgoric acid

8. The Hormone of the Parathyroids[43]

The parathyroid glands secrete a hormone which regulates calcium metabolism. In the absence of sufficient secretion *tetany* sets in. The hormone has not been isolated in the pure form and appears to consist essentially of protein material.

9. The Hormone of the Pancreas—Insulin[44]

Insulin is produced by the islets of Langerhans in the pancreas and regulates the process of carbohydrate metabolism. An inadequate secretion of the hormone causes insufficient carbohydrate utilization and excretion of sugar through the kidney. This disease is known as *diabetes mellitus*. Insulin has been obtained in crystalline form and is a protein with an unusually high sulfur content. This hormone apparently occurs in the organism in combination with zinc. The yields of insulin can be considerably improved when a zinc salt ($ZnCl_2$) is added to the pancreas extract which causes the crystallization of a *zinc-insulin complex*. The combination of the zinc complex with spermin which is known as *protamin-zinc-insulin* is also of interest. The action of insulin in this combination is protracted, the maximum effect occurring about 12 to 24 hrs. after administration while the unmodified insulin reaches its maximum effect in from 2 to 3 hrs. All insulin preparations have to be given by injection. The protamin-zinc-insulin is to be injected only subcutaneously while the unmodified insulin can also be given intravenously or intraperitoneally.

One milligram of a standard preparation of zinc-insulin crystals contains by definition 22 insulin units. One U. S. P. insulin unit will on an average promote the metabolism of approximately 1.5 g. of dextrose.

---

[43] F. Albright, *J. Am. Med. Assoc.*, **117**, 527 (1941). D. L. Thompson and J. B. Collip, *Physiol. Revs.*, **12**, 309 (1932). W. F. Ross and T. R. Wood, *J. Biol. Chem.*, **146**, 49 (1942).

[44] E. T. Waters and C. H. Best, *J. Am. Med. Assoc.*, **117**, 852 (1941).

10. Some Less Well-Known Hormones

The opinion has frequently been expressed and supported in part by experimental evidences that the *thymus gland* and the *pineal gland* secrete special hormones. Neither of these has, however, been isolated and the opinion about their physiological activity is controversial. The thymus gland appears to contain a growth-promoting compound which also exerts certain influences upon the development of the sexual apparatus.

Secretin is the name given to a hormone produced in the duodenal mucosa. It stimulates the outflow of pancreatic juice and bile and appears to be of protein character.

Renin is the term applied to a substance which is present in the cortex of the kidney. This compound has vasopressor activity and produces renal hypertension.

Acetylcholine, $CH_3—COO—CH_2—CH_2—N(CH_3)_3OH$, is responsible for the transmission of nerve impulses from parasympathetic fibers. It is rapidly deactivated at the site of its action by a special enzyme which accomplishes hydrolysis.

Kallikrein, probably identical with *angioxyl*, *vagotonine*, and *padutin*, is claimed to be a blood pressure depressant of protein nature and has been obtained from the pancreas. It may be similar to *carotidin*, a postulated hormone from the carotid gland.

Chapter VIII

MINERAL MATTERS AND OTHER INORGANIC FOOD ADJUNCTS

R. H. Carr

Lafayette, Indiana

I. INTRODUCTION

The rapid expansion in the field of mineral metabolism calls for frequent reviews and interpretations of the new data. Calorific values of food material no longer hold the spotlight, nor does protein metabolism command the attention that it did in the past. Instead there is unusual interest in trace elements, vitamins, vitagens, acid-base equilibria, and minerals in both food and foodstuffs.

There is no pretense on the part of the author of making an exhaustive presentation of the conflicting data as they now exist, but I shall try to interpret what seems most important in order to lessen confusion in the minds of the readers. The literature on the various phases of mineral nutrition has become so great that the continuous citing of references would tend only to confuse rather than to furnish the reader with a perspective for further study. Hence, only the more direct references which may serve to guide further reading will be given.

1. The Elements in Biological Materials

In the days of the chemist Lavoisier (1743–1794) only 26 chemical elements were known. Other elements were rapidly added to the list, but it was not until the time of Liebig (1803–1873) that the importance of minerals in plants and animals was appreciated. Now the number of elements believed to be of importance in biological material only, has increased until 25 have been included. They may be divided for convenience into five groups as follows:

1. The electropositive elements: sodium, potassium, calcium, and magnesium.
2. The electronegative elements: chlorine, phosphorus, and sulfur.
3. Small amounts of two very important elements: iodine and iron.
4. Trace elements: "micronutrients," or possibly "contaminants," cobalt, nickel, copper, aluminum, fluorine, arsenic, bromine, selenium, zinc, and silicon.
5. The organic component elements: carbon, hydrogen, oxygen, and nitrogen. (Also phosphorus and sulfur.)

It will be noted that the trace element group (4) has some elements like selenium, arsenic, and nickel which seem to be present as contaminants; however, under certain conditions they act in a way to stimulate living cells.

2. Definition

A mineral has been defined as a solid or liquid homogenous inorganic substance which is a direct product of nature. The mention of the word "liquid" calls special attention to water as a true mineral even though it is not usually thought of as such. Water is very important in connection with mineral nutrition, as it constitutes about 70% of the human body, 78% of the muscle of a sheep or a steer, and 79% of the weight of a lobster or a snail. This high water content of living tissue was well expressed by Bernard[1] who stated that "all living matter lives in water."

The words mineral or inorganic as applied to mineral metabolism are not very definite, as the term "inorganic" might include ashless compounds like water, carbon dioxide, carbonic acid, nitrous acid, nitric acid, or ammonium carbonate, whereas it is customary to call mineral only that part of food which leaves an ash on burning. In a broad sense these compounds are included in all discussions of living matter as part of mineral metabolism; however, it is the custom to omit them from the mineral element list.

3. Foods and Foodstuffs

It is the usual practice in discussing the feed of animals and the food of human beings to make a distinction in the terms feed or foodstuffs, and food, although the general principles involved are very closely related. This separation in words is designed to call attention to the differences, however, as the concentration of animal foodstuffs is greatly intensified as compared to that of human food. The rapid growth and fertility of most farm animals require a mineral concentration in the milk some-

TABLE 29

INFLUENCE ON GROWTH OF AMOUNT OF PROTEIN AND MINERAL MATTER IN MILK
(By Bunge and associates[a])

Species	Time (days required to double weight)	Per cent in milk of:			Total ash
		Protein	Lime	Phosphoric acid	
Human	180	1.6	0.03	0.05	0.20
Horse	60	2.0	0.12	0.13	0.40
Cow	47	3.5	0.16	0.20	0.70
Sheep	15	4.9	0.25	0.29	0.84
Pig	10	5.2	0.25	0.31	0.80
Dog	9	7.4	0.45	0.51	1.33
Rabbit	6	14.4	0.89	0.99	2.50

[a] E. Abderhalden, *Z. physiol. Chem.*, **27**, 594 (1899).

---

[1] Claude Bernard, *Leçons de physiologie expérimentale.* Paris, 1855.

what in proportion to the time required to double their weight. This is shown in Table 29.

The importance of the mineral content in feed was not realized until attempts were made to feed synthetic diets to experimental animals. Forster,[2] in 1873, fed pigeons and dogs on purified diets with low salt content and noted that the animals died in a few weeks. He partly misinterpreted his data, however, as he had left vitamins out of the ration. Others had studied the effect of the varying salt content of feed, but it was not until the work of Bunge,[3] in 1902, that the mineral composition of the body was shown to parallel closely the composition of the milk of that species. Since the time of Bunge most purified diets for use with experimental animals have been supplemented with about 4% of the minerals in cow's milk.

4. Ash

The residue obtained when organic materials in a feed or animal tissue are burned off is called ash. Ash determination, in addition to other constituents, is used in feed analysis to determine the nitrogen-free extract. The ash may include carbon in the form of carbonate if base-forming materials were in the feed in some excess. The analysis of the ash of a feed gives no clue as to how the mineral occurs combined, so the ash determination tells us nothing about the elements present. Minerals function in the body in definite organic and inorganic combinations and the usefulness of minerals in food depends upon these combinations. Sulfur, for example, is needed in food mainly as a part of the amino acids, methionine and cystine, but the determination of the sulfur content of the ash in a food would give no information as to how this element has been combined. It is necessary, therefore, for the chemist to use other methods than ash analysis to determine the forms in which the element sulfur occurs in the body. The ash determination, however, is worth while, as it furnishes the most rapid and comprehensive way of determining the mineral content of both animal and plant material. Newer methods of making ash determinations by using low heat in an electric furnace have tended to correct some data of losses of mineral elements noted by the earlier workers. The most serious losses of elements in ashing at high temperatures of 800° C. instead of 450° C. are those of sulfur, chlorine, potassium, and sodium. In order to make sure of avoiding losses even at low temperature it is customary to add acids to prevent loss of the positive elements, and alkali to prevent the loss of the negative anions. After the ashing has been completed, the residue contains carbonates, oxides, salts, meta- and pyrophosphates, and sulfates. It would seem that ash determinations would be among the simpler tasks of a chemist, but experience proves that the results, especially from those materials containing a high percentage of oils or waxy compounds such as soybeans, are frequently far from uniform in appearance and weight.

---

[2] J. Forster, *Z. Biol.*, 9, 297 (1873).

[3] G. Bunge, *Textbook of Physiological and Pathological Chemistry.* 2nd English ed., Blakiston, Philadelphia, 1902.

The total ash percentages of a few parts of the human body are as follows: skeleton 83.2, muscle 10.3, skin 1.0, brain 0.7, lungs 0.5, and heart 0.1. The ash of the average adult body is about 4.35%, with four-fifths of the total ash in the skeleton, and only 1% in the organs. It is evident, therefore, that the skeleton is the big mineral storehouse of the body structure.

5. Bound and Metabolic Water in the Body

All of the organic and inorganic compounds of the body, except the salts in the skeleton, are present in either an emulsion, a watery suspension, or a true solution. The water in the living body makes up over 60% of body weight, and most of the tissue contains from 70 to 90%, making the soft parts a very watery substance. In the case of a tadpole weighing about 2 g., which was developed in pure water from a 2-mg. frog's egg, the increase in weight was only water taken on and it was vitalized to become a living being containing less dry matter than the original egg, since some of the egg had to be used to furnish energy. It is believed by many physiologists that part of the water contained in living tissues is "bound" by colloids, being part of a disperse phase in contrast to "free" water. The bound water is so fixed or stabilized as to prevent it from being forced out by pressure. Rubner,[4] in 1922, defined bound water as that part of the water in tissues which did not freeze when cooled to $-20°$ C. Water which is bound in living tissue serves a different, and undoubtedly a more important purpose than free water. For example, certain plants like cactus are enabled to resist drought in summer, and wheat plants resist freezing in the early winter because a large part of all the water present is in the bound form.

There are a few physiologists who maintain that because a small amount of water is bound by colloids it does not warrant the concept of water existing in the "bound" form.

The interesting work on metabolic water by Babcock[5] (1843–1931) paved the way for a better understanding of the metabolic processes of the body which furnish water, other than that consumed directly or taken in as food. When carbohydrates, fats, and proteins are oxidized in the body to furnish energy, water and carbon dioxide are also produced, as noted in the following reactions:

$$\text{Carbohydrates:}\quad C_6H_{12}O_6 + 6\ O_2 = 6\ CO_2 + 6\ H_2O$$

$$\text{Fats:}\quad C_{57}H_{104}O_6 + 80\ O_2 = 57\ CO_2 + 52\ H_2O$$

It can be shown by calculations that 60% of the glucose, about 42% of the protein, and 100% of the fat consumed by animals are oxidized in the metabolic processes to produce a further available quantity of water. Hibernating animals such as groundhogs and squirrels live largely on metabolic water all winter. In these animals the reserves of food, especially the fats, are metabolized and produce sufficient water to meet the animal needs as well as furnish enough extra water to balance the loss in evaporation and respiration. Similar metabolic processes go on in plants.

---

[4] Max Rubner (1854–1932), Professor of Hygiene, University of Berlin.

[5] S. M. Babcock, Wisconsin Agr. Expt. Sta., *Research Bull.* 22 (1912).

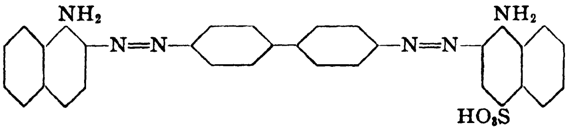
6. Ringer-Locke's Solution of Minerals

An interesting physiological relation among the minerals was noted more than thirty years ago in Ringer-Locke's solution. This solution was developed before the days when much was known about ionization. It was learned empirically that when surviving tissues were immersed in the solution they were able to maintain their physiological activity for a considerable time. This solution contains 0.7% Na^+ and Cl^- and small amounts of K^+, Ca^{++}, and HCO_3^- ions (KCl 0.03, $CaCl_2$ 0.025, $NaHCO_3$ 0.003). When a surviving heart of a cold-blooded animal, such as a frog or turtle, is immersed in a solution of Na^+ and Cl^-, K^+ and Cl^-, or Ca^{++} and Cl^- an abnormal contraction of the heart takes place and it soon ceases to beat. If, however, the heart is placed in a little stronger potassium chloride solution it comes to rest in a dilated condition. Locke showed that the most desirable mineral salt solutions on the hearts of warm-blooded animals were somewhat different from those desirable for the cold-blooded animals. A solution of the following composition was found to be most efficient for warm-blooded animal tissues: sodium chloride 0.9%, calcium chloride 0.024%, potassium chloride 0.042%, and sodium bicarbonate 0.01 to 0.03%.

7. Donnan Membrane Equilibrium

The permeability of a membrane to ions and to the solvent may help to explain the physiological effect of different minerals on animal tissues. The ions on each side must be equal; if they are not equal, a strain in the membrane is developed which interferes in attaining an equilibrium. This unbalance in the distribution of ions is believed to occur frequently in animal tissues and to a lesser extent in plant cells not recently changed by the addition of fertilizers.

A theory has been suggested by Donnan which offers an explanation of this unbalanced situation. He makes use of an aqueous solution of Congo red,



which is a colloidal electrolyte that ionizes completely in contact with sodium chloride. The Congo-red solution is placed inside the membrane bag and is then placed in the sodium chloride solution. The sodium chloride ions diffuse through the membrane, in time, until an equilibrium is reached. The movement of the ions may be shown by the following diagram of the beginning and final condition:

	Membrane	
Start:	(1) Na^+ R^- C_1 C_1	(2) Na^+ Cl^- C_2 C_2
Final:	(1) Na^+ R^- C_1 C_1 Na^+ Cl^- x x	(2) Na^+ Cl^- $C_2 - x$ $C_2 - x$

The aqueous solution at equilibrium has x quantity of sodium chloride passed through the membrane away from the first salt solution. The actual concentration of the Na^+ ions on the left-hand side of the membrane consists of the number originally present, C_1, plus the number diffused, x. If a nondiffusible ion were not present, then at equilibrium the product of the concentrations would be equal:

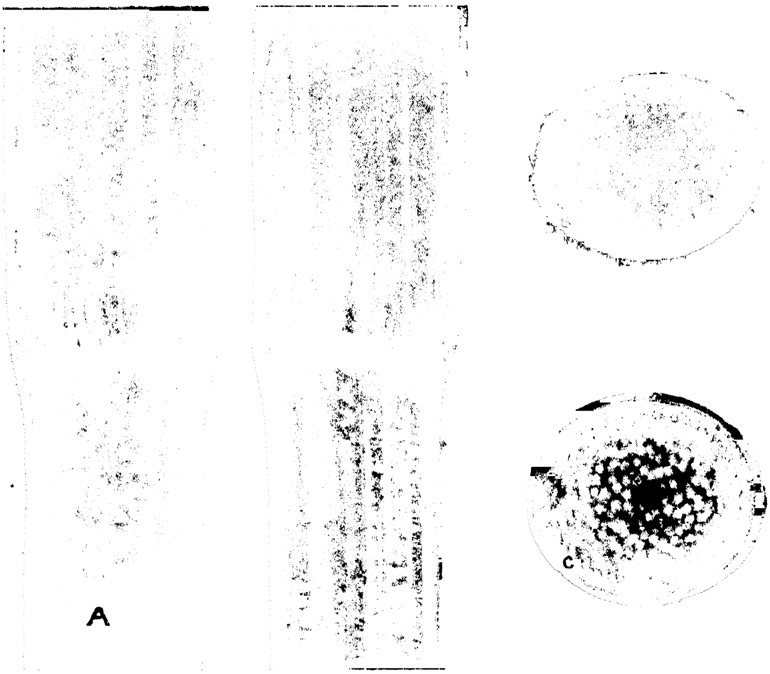


Fig 16-1. Longitudinal section through a node of a stalk showing symptoms of root rots The darkened vascular bundles are stained by a dilute solution of methylene blue which was absorbed by the plant The nonfunctioning bundles have the largest accumulations of iron and aluminum in the zone *B* region. *B*, *C* Relative numbers of functioning vascular bundles in transverse sections of diseased stalks. *B* shows the internode section and *C* a section through zone *A*.[6]

$$\underset{\substack{\text{Left side}\\\text{of membrane}}}{[Na^+]\cdot[Cl^-]} = \underset{\substack{\text{Right side}\\\text{of membrane}}}{[Na^+]\cdot[Cl^-]}$$

---

[6] G. N. Hoffer and R. H. Carr, *J. Agr. Research*, **10**, 801 (1923).

But since a nondiffusible ion is present, the concentration of the Na^+ ions on the left side of the membrane must, as pointed out, be equal to the concentration of both the nondiffusible ion R and the Cl^- present. This means that the concentration of the sodium ion on the left side is greater than the concentration of the sodium ion on the right side, and vice versa for the chloride ions:

$$\underset{\text{Left side}}{[Na^+]} > \underset{\text{Right side}}{[Na^+]} \quad \text{and} \quad \underset{\text{Left side}}{[Cl^-]} < \underset{\text{Right side}}{[Cl^-]}$$

Though there is electroneutrality on each side of the membrane at equilibrium, the difference in concentration of diffusible ions causes a potential difference between the two sides of the membrane. This may account for some of the electrical phenomena exhibited by living matter.

Thus, by the use of the theory Donnan suggested, one can understand how different concentrations of diffusible ions may be present on the two sides of a parchment. There are many other compounds, like sodium proteinate, for example, which would act in the same way to modify diffusion of body minerals.

Mineral metabolism of plants has reached a new refinement in the development of water culture. Fertilizers when added to a soil often produce an unblanced condition in the sap of the plants grown there until a new equilibrium can be established. Acids in soils often make aluminum and iron compounds available to the plant and cause an unbalance of soil solution in the membranes great enough seriously to retard growth. This condition has been shown by Hoffer and Carr[6] to be present in the nodes of corn plants where iron and aluminum deposits have clogged up the membrane (Fig. 16). The iron in the nodes can be easily detected by the red color of the node developed when treated with potassium thiocyanate and hydrochloric acid. The presence of aluminum compounds may be shown by heating sections of the nodes with logwood extracts in the presence of a solution of ammonium carbonate. This treatment causes a blue stain to form in the membrane when aluminum compounds are present.

II. CALCIUM AND PHOSPHORUS

It is desirable to study calcium and phosphorus together, since they are so closely interrelated with each other in body metabolism. Each element, however, has its own definite purpose in the living organism, and its place cannot be taken by another, however scarce that element may become.

1. Calcium and Phosphorus in the Bone

A discussion of the composition of bone appears to be the logical starting place, as about 80% of the phosphorus and over 98% of the calcium of the body are in the teeth and bones. The bone of mammals is made of about 85% tricalcium phosphate, 14% calcium carbonate, and 1% magnesium phosphate. The ash analysis indicates that, approximately, the bone consists of phosphorus 17%, calcium 36%, magnesium 0.8%, and carbon dioxide 5.43%. In addition the bone contains about 50% water, 20% protein, and 4% fat, the ash making up 26%. It is evident from the mineral analysis that the calcium and phosphorus are in about a two to one ratio, varying only slightly with age and diet. The normal daily intake of calcium is about 1 g., but only a part of this can be assimilated because it is in an unavailable form. Excessive phosphorus in the diet will often cause a reduction in the amount of calcium absorbed on account of the formation of insoluble calcium compounds. Calcium should be present in about one or one and one-half parts to two parts of phosphorus. An incorrect proportion of calcium and phosphorus intake may be as harmful as a deficiency of either. The chemical composition of teeth is like that of bone, differing in the pulp, enamel, and dentine. The enamel is noted for hardness and low water content.

2. Calcium and Phosphorus in Blood

Nearly all the calcium of the blood is in the serum; the blood cells are almost devoid of this element. The concentration of calcium in the serum is normally about 10 mg. per 100 ml. Calcium is present in the serum in two different types, known as diffusible and nondiffusible. The diffusible type is that portion of the serum calcium which can pass through an artificially prepared membrane, whereas the nondiffusible type, which comprises 50 to 60% of the blood calcium, cannot pass through the membrane because it is combined with serum albumin. It is the diffusible kind (that is, the active calcium in the ionized condition) that is combined largely as phosphates and bicarbonates. It has been shown by Taylor and Russell[7] that the diffusible calcium of hens remained nearly constant at 5.4 mg. during the high egg production period whereas the *nondiffusible* calcium contrary to expectation, rose from about 6.4 mg. in nonlaying hens to 16.1 mg in the egg-laying period. The level of blood calcium is controlled not by the food, but by a hormone from the parathyroid glands. If the parathyroid glands are removed by operation, the calcium level of the blood soon drops. When these glands are unusually active, there is an excessive withdrawal of calcium from the bones, resulting in a loss of calcium from the body through an excessive excretion in the urine. Serum calcium plays an important role in the body by maintaining a balance of calcium ions in the acid-base equilibrium, and it is also needed in the clotting of blood. Tetany or possible rickets may occur when the blood calcium is low. Milk fever may occur in a cow when the demand for excess calcium is unusually great as at the time a calf is born. The calcium to phosphorus ratio is maintained constant in the blood as well as elsewhere in the body, varying from 35 to 40 mg. of phosphorus in the blood cells in organic forms.

3. Rickets

In a broad sense any factor which prevents calcium and phosphorus retention in the body in adequate quantities causes rickets. Instances of rickets have been reported as due to the formation of insoluble phosphates of lead, iron, magnesium, beryllium, or tellurium. The cure of rickets with Vitamin D has made us "vitamin D conscious." Just how this is done is not known, but the vitamin D acts by restoring phosphorus and calcium of the blood to a normal amount. In the past it was the custom to overemphasize the loss of calcium as the cause of rickets while phosphorus was overlooked; it has been shown that rickets can be produced by foods lacking in phosphorus. Whatever the cause may be, the result is a decreased ash in the bones. In the more advanced stages there are external symptoms like stiffness, lameness, paralysis, and abnormalities of bony growth which are characteristic. The destruction is sometimes spoken of as low-phosphorus rickets, low-calcium rickets, or true rickets. It is very common to find rickets in pigs and calves, as well as in human beings, and other animals are subject to the disease, as cases have been reported in dogs, sheep, and horses. In fact some form of rickets seems to occur in all species when the calcium-phosphorus ratio has been seriously disturbed. A low blood calcium has been more frequently noted than a low blood phosphorus in calves whereas in pigs a low phosphorus content is common. The work with calves was reported by Bechdel and associates[8] and that with pigs by Bethke and coworkers.[9]

---

[7] M. W. Taylor and W. C. Russell, *J. Agr. Research*, **51**, 663 (1935).

4. Urinary Calculi

The conditions which cause stone formations have baffled medical authorities for a long time. Abnormally high concentrations of calcium and phosphorus in the food, blood, and urine provide the natural constituents for the production of insoluble phosphates, and it is to be expected that stones might form in the kidney or bladder and smaller granules lodge in the ureters. The wonder is that stones do not form more often. In addition to calcium- and phosphorus-bearing crystals, oxalates, carbonates, magnesium compounds, and organic compounds are common urinary components.

Other types of stone formations such as gallstones are often spoken of as being composed mainly of cholesterol and bile pigments, but more detailed investigation shows that they also contain large amounts of calcium, magnesium, phosphates, and carbonates. These stones often have a waxy center coated with concentric layers of calcium urate and phosphate in a rather definite pattern. It has been shown by Albright and coworkers[10] that many of the stones are associated with an increased calcium content in the serum, a condition common in renal rickets of children. It has been shown by Pontius, Carr, and Doyle[11] in a study of stone formation in sheep that stones were produced in considerable quantity in the bladder, kidney, and lumen when the sheep were fed heavily on alfalfa or clover containing much calcium together with a grain ration containing added bran with a high phosphorus content (Fig. 17). In contrast to this no accumulation of stones was found in sheep which were fed grass or root crops high in potassium and bran which produced soluble phosphates. This result seems to indicate that a measure of control can be had through the choice of feeds

---

[8] S. I. Bechdel, K. G. Landsburg, and O. J. Hill, Penna. Agr. Expt. Sta., *Tech. Bull.* **291** (1932).

[9] H. E. Bethke, B. H. Edgington, and C. H. Kick, *J. Agr. Research*, **52**, 149 (1933).

[10] F. Albright, P. C. Baird, O. Cope, and E. Bloomberg, *J. Am. Med. Sci.*, **187**, 49 (1934).

[11] B. E. Pontius, R. H. Carr, and L. P. Doyle, *J. Agr. Research*, **42**, No. 7 (1931).

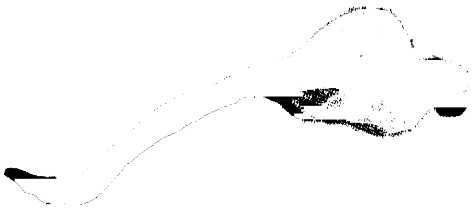


Fig 17.—Calculus lodged in the lumen of the *processus urethrae* (worm) of a Rambouillet ram.[11]

5. Feed Variations in Calcium and Phosphorus

There is a wide variation in the calcium and phosphorus content of both the roughages and concentrates of animal feeds, as well as human foods. Although the detailed figures are available in many reports, it may be worth while to point out how very low is the calcium content of corn, wheat, oats, wheat bran, gluten feed, and soybeans. The calcium content of all those mentioned is under 5 lbs. per ton, with corn even less than 1 lb. per ton, in contrast to clover and alfalfa hay, which have 23 and 24 lbs. of calcium per ton, respectively. In regard to the phosphorus content of feed, however, somewhat the opposite condition is noted as corn fodder, alfalfa, clover, and timothy hay all contain less than 5 lbs. of phosphorus per ton, whereas concentrates like wheat bran and cottonseed meal contain about 24 lbs. of phosphorus per ton with dry skim milk only about 5 lbs. less per ton. It will be noted that the stock feeder usually has a wide choice for making a balance of these necessary elements without buying mineral supplements

6. Food Variations in Calcium and Phosphorus

A similar review of these two elements in the common human foods reveals that the extremes are not so wide; especially is this the case with cheese. Swiss cheese is very high, as it contains about 1086 mg. of calcium per 100 g. and nearly 812 mg. of phosphorus in the same amount, which is unusual. Other common calcium foods are molasses 258, kale 197, milk 120, navy beans 158, and eggs 65 mg. per 100 g. Other phosphorus food sources are nuts 350–450, roast veal 287, canned fish 250–275, chicken 210–270, fresh ham 269, white of egg 224, and lamb 200–210 mg. per 100 g. It will be evident that all meats and eggs have a high phosphorus content. It is difficult to determine the amount of phosphorus used in a food because under normal conditions it is absorbed and removed from the intestinal tract. The amount of calcium found in feces, however, is much greater than the amount of phosphorus because the calcium is bound with some fatty acids as well as with phosphorus. This normal condition is frequently reversed in cases of rickets with the phosphorus in excess of the calcium. Some of the phosphorus in seeds is present as phytin, the calcium-magnesium salt of inositol hexaphosphate. This phosphorus compound is not well utilized by children (or rats). It has been shown by McCance and Widdowson[12] that 20 to 50% of the phytin in cereals is excreted by human beings, in the feces, although the evidence indicates that hogs and cattle can make much better use of this compound. The availability to children of calcium in milk has been shown to be greater than that contained in leafy vegetables, as might have been expected. It is possible that this is due partly to the interference of the fiber content of vegetables, although the calcium found in kale, which is nearly free from oxalic acid, was well utilized, whereas that in spinach containing some oxalic acid was poorly assimilated.[13]

III. MAGNESIUM

Magnesium is widely distributed in food material and in the body, but its physiological significance is still much of a mystery. It is widely used chemically for its cathartic and alkalizing effect as well as for its diuretic action.

1. Deficiency and Excess of Magnesium

Studies by McCollum and associates[14] of the effect of a deficiency of magnesium in a diet have indicated that there are two distinct phases in its effect; one is manifested in the dilation of blood vessels and overexcitability, the other effect is malnutrition and kidney damage. Other evidences of animal malnutrition caused by magnesium deficiency are a rough coat, loss of hair, and diarrhea. High magnesium intakes are usually without harmful effects in experimental animals, up to 4 g. of magnesium having been given per 100 g. of feed.

2. Antagonistic Action of Calcium and Magnesium

The chief body storage deposits of magnesium are in the bones and muscles. The bone ash varies somewhat in different species but will average between 0.5 and 0.7%. It has been shown in a study of the bones of rats that the magnesium decreases as the calcium increases and that the one element cannot take the place of the other, even though they are closely allied. Meltzer and Auer[15] have shown that calcium overcame certain inhibitory effects on body function caused by the injection of magnesium salts. It was later shown that injection of calcium salts caused a seriously increased output of magnesium in the urine. According to Palmer and associates[16] the injection of magnesium sulfate into cattle fed on a low phosphorus ration caused a serious loss of calcium. Later experiments by the same men showed that magnesium limestone did not seem to interfere in bone formation or disturb calcium retention when fed to rats, chickens, and pigs on a ration containing a plentiful supply of phosphorus.

---

[12] R. A. McCance and E. M. Widdowson, *Biochem. J.*, **29**, 2694 (1935).

[13] M. L. Fincke and H. C. Sherman, *J. Biol. Chem.*, **110**, 421 (1935).

[14] E. V. McCollum and E. R. Orent, *J. Biol. Chem.*, **92**, 30 (1931).

[15] S. J. Meltzer and J. Auer, *Am. J. Physiol.*, **21**, 400 (1908).

3. Magnesium Supply in Foods

Milk and vegetables are the two main sources of magnesium in the human diet. The green leaf vegetables have magnesium as a part of the chlorophyll molecule, consisting of about 0.22%. Chlorophyll, a complicated structure with its magnesium atom as the only metallic element present, is believed to be a very active constituent of green leaves, and an important aid in the treatment of anemia. Food sources of magnesium include the following: wheat germ containing 511 mg. per 100 g.. honey 386, endive 380, kohlrabi 370, dried Lima beans 188, peanuts 180, and dried peas 149. It is stated by Sherman[17] that the average daily human consumption of magnesium in the United States is about 0.27 g. This quantity would be available in a 26-g. portion of endive, or a 40-g. portion of fresh peas. In a country like Finland where more leafy vegetables are used, it is calculated that an average of 0.74 g. is used per person per day. The normal breast-fed infant would consume about 0.70 g. per day. The actual requirement of the body for magnesium has not been extensively studied, but it is believed that the average American intake is sufficient.

IV. SODIUM, POTASSIUM, AND CHLORINE

The sodium and potassium compounds in food lend themselves to study together, not because of their chemical similarities but because of their differences in cell activities and locations. Sodium is found especially in the fluids outside the cells, whereas potassium is located mainly within the cells. The contrast is great also between the large content of potassium in all plant cells and the small amount of sodium contained in plants; thus, blue grass may have an 18 to 20 times greater potassium than sodium content. Potassium is present in the body mainly as compounds of phosphate, together with chlorides and bicarbonates, while sodium is mainly in the form of chloride and bicarbonate. Again, a contrast is noted in the degree of body tolerance. Excess potassium consumption of about 25 g. may have serious consequences as a diuretic, since there is no storage facility for this element. On the contrary, 36 to 38 g. of sodium chloride may be consumed in one day without ill effects. Finally, sodium is concerned in the regulation of body neutrality, as it comprises about 93% of the blood-serum base. These points of difference will serve to emphasize how completely these two necessary mineral elements play a separate role in the body.

---

[16] L. S. Palmer, C. H. Eckles, and D. J. Schutte, *Proc. Soc. Exptl. Biol. Med.*, **26**, 58 (1928).

[17] H. C. Sherman, *Chemistry of Food and Nutrition.* Macmillan, New York, 1937.

1. Bunge Theory of the Sodium and Potassium Ratio

Bunge,[18] in 1902, noted that sodium chloride was always desired with vegetables which contain a high amount of potassium Herbivora living on grasses are accustomed to gather around salt licks for salt which was regarded by Bunge as essential for life and not just a condiment. He came to believe that the sodium-potassium ratio was the important factor in nutrition balance, and that an excess of either caused an excretion of the other. This work has been studied by many investigators since that time and it has been found that when only moderate quantities of both are used the retention is not much affected. When cattle are given free access to salt there is no evidence of any appreciable change or variation in the output of potassium. Gamble[19] has shown that in urine of maximum concentration the sodium and potassium were additive, so that the urine had the same concentration of cations whether the mineral used was sodium, potassium, or any mixture of the two.

When growth is taking place rapidly there is need of excess potassium in the body over that of sodium such as is supplied in milk, where the sodium-potassium ratio equals 1:2.5 by weight, whereas for adults the ratio of intake averages only 1:1.5 by weight. In the case of herbivora the roughage used has a wide sodium-potassium ratio of about 1:18 and is an alkaline feed. Salt, in addition to cereal grains which produce an acid balance, would be needed to supply the chlorine to make the necessary hydrochloric acid. The carnivora, on the other hand, live on a meat diet with a high potassium content and that together with the sodium in the body fluids furnishes about the same ratio of sodium-potassium as is found in milk, which seems to be even more than adequate; hence, relatively little sodium chloride would be needed for meat-eating animals.

2. Chlorine

Chlorine is found in the body in large amounts both within and without the tissues and cells. Nearly one-third of the total chlorine content is found in the skin and tissue beneath. The blood cells contain about one-half the amount present in the plasma. Much of the chlorine of the body is present in organic combinations; in some cases this amounts to as high as 20% of the total. It is the chlorides of the blood which furnish the acidic ions, and these largely control the acid-base balance.

3. Sodium Chloride

The average person living in the United States has become accustomed to use about 10 g. of sodium chloride per day, which is about half of that used in Europe. Much of this is used as a condiment, the nutrient requirement being much less than that amount; the excess is excreted in the urine in the form of sodium chloride. The salt water intake relation is important in the use of both sodium and chloride, as in the case of low salt with liberal protein intake the volume of urine is controlled by the need for urea excretion. "Water intoxication" results when low salt intake accompanies high water intake, whereas on a high salt and low water intake a "salt fever" develops for the osmotic pressure of the fluids of the body becomes greater. In kidney disease the organ loses its ability to excrete even a normal amount of chloride ions, and this may result in edema, or the formation of excess water in the tissues. It has been shown by Gamble and Ross[20] that sodium ions are more important than chloride ions in regulating the extracellular fluid, because sodium is not replaced by any other ion whereas the chloride loss may be replaced by bicarbonate ions. In brief, sodium chloride functions in two important ways: First, it governs the osmotic pressure of the fluids surrounding the cells, and, second, it maintains the fluids "on the alkaline side" through control of the acid-base balance.

---

[18] G. Bunge, *Textbook of Physiological and Pathological Chemistry.* 2nd English ed., Blakiston Philadelphia, 1902.

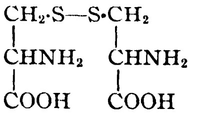
[19] J. L. Gamble, *Bull. Johns Hopkins Hosp.*, **61,** 174 (1937).

V. SULFUR

Sulfur, although classed as a mineral element, is so completely bound with organic groups as to make it appear as an organic compound; hence its study is very complicated. Sulfur is present in every plant and animal cell. The wheat plant can start with sulfur in the inorganic form as calcium or ammonium sulfate and form a protein, gliadin, that has a sulfur content of about 1%, but the animal cannot use the inorganic form of the element.

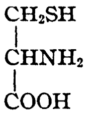
1. Sulfur-Containing Amino Acids

Cystine, cysteine, methionine, and ergothionine are the sulfur-containing amino acids found in proteins available to animals. It has been shown by Rose and associates[21] that methionine is an essential amino acid for the growth of rats, but that cystine is not essential. When, however, a synthetic mixture was fed containing 8% casein as the form of protein, together with either cystine or methionine, growth was produced. It is possible, therefore, for certain nonsulfur-containing compounds to furnish what is essential in amino acids. The following structures show how sulfur is combined in (I) cystine, (II) cysteine, and (III) taurine derived from cysteine:



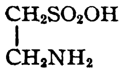
$$\begin{array}{ll} CH_2\cdot S{-}S\cdot CH_2 & \\ | \qquad\quad | & \\ CHNH_2 \;\; CHNH_2 & \\ | \qquad\quad | & \\ COOH \;\;\; COOH & \end{array}$$

I



$$\begin{array}{l} CH_2SH \\ | \\ CHNH_2 \\ | \\ COOH \end{array}$$

II



$$\begin{array}{l} CH_2SO_2OH \\ | \\ CH_2NH_2 \end{array}$$

III

It will be noted that the SH group present in cysteine has been found to be characteristic, as it gives the nitroprusside reaction not given by cystine or native protein. It is not given, for example, in fresh egg protein but develops remarkably on incubation for 30 to 35 hrs. The action of bacteria in the intestinal tract is responsible for many sulfur compounds being formed, such as ethyl (CH_3CH_2SH) and methyl (CH_3SH) mercaptans, thiosulfates ($Na_2S_2O_3$), and indoxyl ($C_6\underbrace{H_4NHCH:C}OH$) together with hydrogen sulfide. If the sulfur derivatives, however, are absorbed into the blood, many of them will be rapidly oxidized to sulfates and be excreted in the urine as sodium and potassium sulfate.

---

[20] J. L. Gamble and G. S. Ross, *J. Clin. Investigation*, **1**, 403 (1925).

[21] W. C. Rose, K. S. Kemmerer, M. Womack, E. T. Mertz, J. K. Guinther, R. H. McCoy, and C. E. Meyer, *J. Biol. Chem.*, **114**, lxxxv (1936).

2. Sulfur in Brain, Muscle, and Blood

The sulfur content of the body is distributed in all parts. The muscles contain about half of the total, while the bones and skin each have one-eighth of the total amount. The sulfur content of the muscle is about one-fourth of one per cent. This is composed mainly of protein sulfur, inorganic sulfur, and a small amount of lipid sulfur. Sulfur comprises 0.50% of the solids of the brain, distributed in both the gray and white matter. The gray matter has approximately 15% and the white 30%; however, the amount of gray matter is about double that of the white. Only a small amount of lipid sulfur is present. The blood normally contains about 1.2 mg. per 100 ml. of inorganic sulfate of blood plasma, in addition to 2.8 mg. of sulfates from conjugated proteins.

3. Sulfur Content of Foods

Most of the common foods high in protein are also high in sulfur, as most proteins contain about 1% sulfur (varying from 0.38% in legumin to 1.62% in egg albumin, and averaging 1%). The following representative foods are noted in order of decreasing sulfur content: wheat germ 325 mg. per 100 g., cheese 264 mg., kidney beans 227 mg., peanuts 224 mg., lean beef 230 mg., egg white 216 mg., and cabbage 173 mg. There is a close relation of nitrogen to sulfur in the proteins, the ratio being approximately 16 to 1. In the growth of wool or hair the demand for both nitrogen and sulfur is heavy, as wool contains about 3.55% sulfur and red hair contains about 5%. Extensive experiments in Australia have shown that the wool clip of lambs was considerably increased when blood meal, a protein of high cystine content, was supplied in addition to the ration furnished by the pasture.

VI. IRON

Although the iron content of the body is very small, less than 0.004%, it has an interesting physiological history. Sydenham,[22] in 1664, had shown that certain solutions of iron salts, known as chalybeate water, given to chlorotic persons would restore pink color to their cheeks. It was not until the 19th century, however, that rational use of iron compounds was made for those suffering with anemia. Ordinary foods, with the exception of milk, usually contain all the iron needed, and it is present in suitable form, but when physiological disturbances occur or the intake of iron becomes insufficient, anemia may result. (Cattle feeding on certain grasses in Florida have become anemic.) Iron functions in the body mainly to cause the foods to oxidize after the oxygen is carried to the various tissues. If the small amount of iron present is not in an available form and well buffered, the hemoglobin of the blood may not be able to produce extensive oxidation.

---

[22] T. Sydenham, *The Whole Works of That Excellent Physician, Dr. Thomas Sydenham the 8th* Edited by J. Pechey, London, 1722, p. 321.

1. Organic and Inorganic Forms of Iron

The term "masked iron" was introduced by Molisch[23] in 1891 to call attention to the fact that iron exists in the body in various forms. The term "organic iron" has been used extensively to contrast its action with that of "inorganic iron." The latter form refers to the easily ionized ferrous and ferric salts of both organic and mineral acids, whereas organic iron relates to forms like hematin and hemoglobin, complex combinations of iron which do not produce ferric and ferrous ions. Iron citrate, for instance, acts as an inorganic type of iron, although, broadly speaking, it too is a complex form of iron.

2. The Formation of Hemoglobin

The length of life of the hemoglobin contained in the blood cells is short—only five or six weeks. During its decomposition the hematin of hemoglobin is changed into bilirubin, and an iron complex together with other pigments which finally reach the liver to be excreted as a bile compound. Hemoglobin tests are frequently made in cases of suspected anemia to see how rapidly the cells are destroyed and renewed. The hemoglobin content should be between 12 and 16 g. per 100 ml. of blood (the average for man is 15.6 and for women one per cent less), and when only 7 to 10 g. are present there is indication of severe anemia. Under normal conditions when the hemoglobin is undergoing destruction the iron may be used again to form hemoglobin, but under diseased conditions the decomposition is too rapid and the processes of cell renewal are too slow to prevent an anemic condition in the body.

3. Nutritional *vs.* Pernicious Anemia

The term nutritional anemia relates to that form caused mainly by the lack of iron as well as the associated copper compound, in the food. Since it is an iron deficiency and milk is very deficient in iron, nutritional anemia is likely to occur during the nursing period. The great deficiency of iron in milk is partially compensated for by the larger percentage of iron in the body at birth than at any time later, indicating that nature made an effort to store iron in the body since it is not provided in milk. This store of iron, however, does not seem to be sufficient in cases of premature birth or in the case of large litters of animals such as pigs, where the demand is greater than the supply. The term pernicious anemia relates, not to a lack of iron as in the other anemia, but rather to a derangement of the essential mechanism by which red blood corpuscles can be formed or activated. The feeding of iron or of iron and copper compounds in this kind of anemia produces little benefit to the patient. Only a combination of iron compounds with extracts of liver or stomach tissue is effective in prolonging life, and no cure has been effected even when the treatment is continued for years. Minot and Murphy[24] have shown that pernicious anemia in the early stages can be treated successfully by the use of large amounts of liver or water extracts of liver. This produces an active substance which normal persons produce by gastric digestion of muscle tissue. This form of anemia is not found in animals. The information which led to a better understanding and dietary cure of this pernicious form of anemia resulted in the Noble prize award in medicine to Whipple, Minot, and Murphy in 1926.

---

[23] A. Molisch, *J. Chem. Soc. Abstracts*, **64** (Part 2), 484 (1893).

4. Iron Content and Its Relative Value in Foods

There is apparently need for distinguishing between total iron present in foods and that part of the iron which is in a form useful in hemoglobin formation in blood. Those common foods which contain total iron in considerable quantities are: beef liver 8.4 mg., raisins 4.0–6.0 mg., beets 2.90 mg., eggs 2.52 mg., navy beans raw 8.2 mg., navy beans cooked 2.0 mg.,and spinach 4.0 mg. raw and 1.5 mg. canned. Elvehjem and associates[25] have shown that the total iron in a food is unimportant as compared to that part of the iron which is in an available form. Thus, inorganic iron salts are completely utilized but hematin iron is not available; liver, heart, and soybeans contain highly available iron as compared to the form of iron in wheat, oats, and tissue from muscle. Other workers, Bing, Benes, and Remp,[26] differ in their reports of the value of hematin iron to rats, finding it used by some of the animals and not by others. It is indicated by different investigators that, since available iron content is somewhat variable in different foods, it is often safest to rely upon the total iron content until more definite information is available. Excess iron salts in a ration have been shown to be not only useless, but in some cases detrimental, as the excess interferes with phosphorus utilization by the formation of insoluble phosphates, and may result in the development of rickets.

VII. IODINE

The first real evidence that iodine is a part of the body tissues was found in 1895 when Baumann[27] showed that iodine is a necessary constituent of the thyroid. Kendall,[28] in 1915, succeeded in preparing the active constituent of the thyroid in crystalline form and he called it thyroxine, a material which contained as much as 60% iodine by weight. The chemical nature of thyroxine was elucidated by Harington and Barger[29] in 1928. The structure of thyroxine is as follows:

---

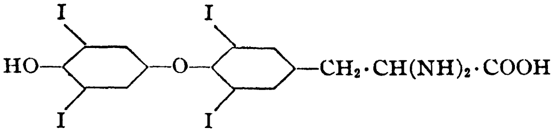
[24] G. R. Minot and W. P. Murphy, *J. Am. Med. Assoc.*, **87**, 470 (1926).

[25] C. A. Elvehjem, E. B. Hart, and W. C. Sherman, *J. Biol. Chem.*, **103**, 61 (1933)

[26] F. C. Bing, F. A. Benes, and D. G. Remp, *J. Biol. Chem.*, **114**, x (1936).

[27] E. Baumann, *Z. physiol. Chem.*, **21**, 319 (1895–1896).

[28] E. C. Kendall, *J. Am. Med. Assoc.*, **64**, 2042 (1915).



It is evident that thyroxine is a tetraiodo derivative of tyrosine and phenol and is an amino acid. The amount of iodine in the thyroid gland varies with age. It is reported to be about 0.10 mg. in a newborn infant and 15 mg. in an adult. In cases of goiter the iodine content is much less than normal and may average less than 1 mg. Although most of the iodine in the body is located in the thyroid gland, it is not all there, but is found in traces all over the body, the total iodine amounting to about 20 mg.

1. The Thyroid Gland

Goiter, which is an enlargement of the thyroid gland, exists in two forms. One is called endemic goiter and the other is called exophthalmic goiter. The former is caused by a deficiency of iodine intake and a relatively mild deficiency of thyroid activity; the latter is hyperthyroidism associated with an excess of thyroid secretion. Goiter is revealed in calves and sheep by an enlargement of the gland, by hairlessness in young pigs, and by leg weakness in colts. In human beings the most critical periods of danger of goiter are puberty and pregnancy, especially the latter if twins are born.

Gudernatch,[30] in 1914, showed how the removal of the thyroid tends to arrest sexual development and change the growth of tadpoles. When the thyroids were removed from a certain number of tadpoles of the same hatch, those that had the glands removed grew larger but did not become frogs like others of the same hatch. After a period of thyroid feeding, however, the normal change to frogs took place.

2. Goiter an Area Problem

In the United States only a few regions are troubled with an iodine deficiency in the soil, notably the Great Lakes region and the Northwest area. Montana has been severely affected by iodine deficiency with the result that before the need for iodine treatment was known there were large losses from hairless pigs and deaths of cattle and sheep from goiter. There are also regions in the Alps, Pyrenees, and Himalayas and near-by territory where thyroid failure is common and goiter very prevalent. A group of dwarfs called cretins have developed there by reason of either the congenital absence of the thyroid gland or the destruction of it by some disease. When the diet of a people, however, contains some sea weeds which are rich in iodine as does the diet of the inhabitants of Japan, goiter is rare or unknown.

---

[29] C. R. Harington and G. Barger, *Biochem. J.*, **21**, 169 (1927).

[30] J. F. Gudernatch, *J. Am. Anat*, **15**, 431 (1914).

3. Sources of Iodine in Foods

Iodine, unlike iron, is effective in the prevention of goiter in any form, as in sodium or potassium salts, or in organic form such as is found in sea weeds, fish, and fish oils. The following sea foods are good sources of iodine: cod-liver oil 3000 to 12,000 parts per billion, cod liver 7680, cod fish 5340, oysters 1800–3600, salmon 570–2000, and shrimp 1100. These values are average reports from the U. S. Bureau of Fisheries. The approximate iodine content of some grains and vegetables from nongoiterous regions are: wheat 4–8 parts per billion dry matter, oats 25–175, potatoes 225–230, cabbage 780, radishes 950, and milk 575. The daily iodine requirement for the adult is not definitely known, but it has been estimated to be between 0.04 and 0.08 mg. With such small daily requirements it is a wonder that any area could be found which would not supply these traces of iodine in food supplies.

VIII. MICRONUTRIENTS

1. Manganese

Manganese and zinc are present in all animals and plants and are considered to be essential in nutrition, at least for the smaller animals studied. It is evident from experiments with rats and dogs that manganese is necessary for reproduction. It is suggested by Orent and McCollum[31] that its function is concerned with the regulation of the sex glands and possibly with the development of the pituitary gland. If conception occurs on rations low in manganese, the young are usually born very weak or dead. Evidence[32] is available to show that lack of manganese is the cause of perosis or "slipped tendon." This is a deformity of the tibial-metatarsal joint of young chickens. The effect of lack of manganese is even more pronounced on plant growth than on animals. The plants soon became etiolated. McHargue[33] has shown the critical need of the plant for manganese; Brewer and Carr[34] have demonstrated how detrimental is the manganic form, and how beneficial is the manganous form of this element in the soil to the growth of plants. Peterson and Skinner[35] have reported on the manganese content of a variety of foods, and it is noted that where manganese is most abundant the seeds and leafy parts of the plants are better developed. The following foods have relatively abundant supplies of manganese: blueberries 4.4 mg. per 100 g., pecans 3.48 mg., graham bread 3.2 mg., dried peas 2.80 mg., and navy beans 2.50 mg. It has been shown that manganese is readily taken up by the plants when manganese fertilizer is added to the soil.

---

[31] E. R. Orent and E. V. McCollum, *J. Biol. Chem.*, **92,** 651 (1931).

[32] H. S. Wilgus, Jr., L. C. Norris, and G. F. Heuser, *Science,* **84,** 252 (1936).

[33] J. S. McHargue, *J. Am. Chem. Soc.*, **44,** 1592 (1922).

[34] P. H. Brewer and R. H. Carr, *J. Soil Sci.*, **23,** 165 (1927).

[35] W. H. Peterson and J. T. Skinner, *J. Nutrition,* **4,** 419 (1931).

2. Zinc

All living tissues contain zinc in quantities of about one-half the iron content. A normal diet includes about 15 to 20 mg. of zinc daily, which is plenty for the body needs. It is not dangerous when taken in some excess by the animal even though it is stored in the body. It is easier to prove that zinc is indispensable to plants than to animals, as Dickey and Blakmon[36] have shown that the lack of zinc is responsible for the formation of little leaf, crinkling of leaves, and chlorosis of peach trees. These disease conditions are overcome by the application of zinc sulfate to the soil. It was shown by Hubbell and Mendel[37] that as little as 0.005 mg. of zinc per day added to an otherwise complete diet was enough to enable mice to grow and thrive. To plan an animal experiment to prove the need of such traces is a difficult matter, as variations in such quantities are hard to detect.

One of the best food sources of zinc is oysters, which contain about 40 mg. per 100 g.; fruits and vegetables have only 3 to 5 mg. in 100 g.

3. Copper

During recent years copper and iron have assumed a new importance in our knowledge of human metabolism. It has been known for a long time that milk is low in iron and that animals limited to a milk diet develop nutritional anemia. In 1928 Hart and associates[38] demonstrated that when copper salts were added to a milk diet the blood count and hemoglobin content were greatly increased and rats that had developed nutritional anemia recovered. Thus a definite physiological function in the animal body has been assigned to copper, since the addition of copper and iron to a milk diet protects against nutritional anemia. It has been shown frequently that when both iron and copper are given to one group of anemic infants, and only iron to another, a much more rapid recovery is made by the group receiving both elements.

Many animals are born with an extra supply of copper stored in the liver; it has been shown that nearly eight times as much copper has been found in the newborn calf liver as is found in the adult animal's liver. The quantity stored in the pig and rat livers of the newborn has also been reported to be at least double that of the full-grown animals. Copper does not combine with the hemoglobin molecule to act directly any more than iron combines with the chlorophyll molecule, but both copper and iron probably function by accelerating or originating reactions without becoming a part of the molecular structures.

Copper in Foods

Copper has been found to be widely distributed in foods. It was shown by Lindow and coworkers[39] that leafy vegetables contain about 1.2 mg. per kg., roots and tubers

---

[36] R. D. Dickey and G. H. Blakmon, Florida Agr. Expt. Sta., *Bull.* **304** (1940).

[37] R. B. Hubbell and L. B. Mendel, *J. Biol. Chem.*, **75,** 567 (1927).

[38] E. B. Hart, H. Steinbock, J. Waddell, and C. A. Elvehjem, *J. Biol. Chem.*, **77,** 797 (1928).

[39] C. W. Lindow, C. A. Elvehjem, and W. H. Peterson, *J. Biol. Chem.*, **82,** 465 (1929).

1.4 mg., and fresh fruits 1.0 mg., while nuts contain much more, 11.6 mg., calves liver 44.1 mg., oysters 30.7 mg., and meat only 1.0 mg. The amount of copper in plants is closely related to the copper contents of the soil, especially where copper fertilizers or sprays have been added, but the addition of extra copper salts to feeds has little effect in increasing the copper in milk or eggs. Allison and associates[40] have shown that when copper is deficient in soils, as occurs in certain soils in the Everglades, Florida, the addition of copper salts as fertilizers increases crop production to a great extent.

4. Fluorine

Fluorine is distributed in all animal tissues, but is especially concentrated in the bones, teeth, and skin. McCollum and associates,[41] in 1925, made an extensive study of the effect of this element on rats and found that only 0.02% of fluoride added to their diets caused a chronic poisoning which affected the teeth. The addition of fluorine caused the teeth to be brittle, opaque, and white, and the enamel gradually took on a

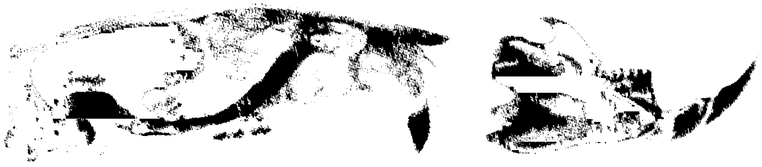


Fig 18 —Normal skull of rat fed on diet without fluorine

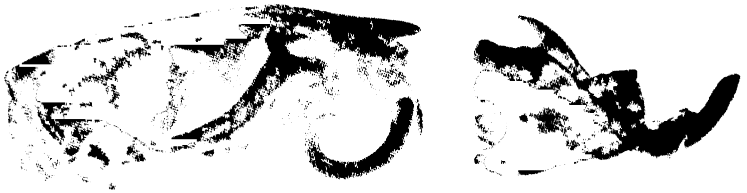


Fig 19 —Thickened skull with elongated and chalky upper incisors caused by excessive fluorine intake Photographs of Figs 18 and 19 by courtesy of the N. Y. State College of Agriculture.

---

[40] R V Allison, O. C. Bryan, and J. H Hunter, Florida Agr. Expt Sta , *Bull* **190** (1927)

[41] E. V. McCollum, N. Simmonds, J. E. Becker, and R. W. Bunting, *J Biol. Chem.*, **63**, 553 (1925).

mottled appearance. The poisoning effect appeared to be caused by the accumulation of fluorine in the tissues and elsewhere. When fed to the hen the fluorine content of the yolk of eggs is increased, but no similar increase has been noted in the milk of cows when fluorine was added to the feed. A mineral supplement sometimes added to feed is apatite or rock phosphate $3Ca_3(PO_4)_2CaF_2$, which contains about 3.5% of fluorine. When this substance is added, there is usually developed a considerably altered bone structure abnormal in size and shape and of brittle texture. Under the same fluorine treatment the upper incisor teeth often overgrow, become curved and white in color, and look like tusks.

Fluorine Areas

An extensive study of 300 fluorine areas of the United States has been reported by Dean[42] and it is noted that about one-fourth of these areas occur in the state of Texas. He found that people living in these territories have the mottled teeth condition, occurring especially in the permanent teeth for the first eight years of life. This condition is correlated with the quantity of fluorine in the drinking water, starting at 0.9 mg. per liter of water and increasing; it is noted that at 2.5 mg. per liter 75% of those drinking the water show the fluorine effect in the teeth, and at a concentration of 6 mg. per liter almost 100% show the effect.

In addition to the detrimental effects of fluorine reported from Texas, there have been some remarkable areas noted there in which a lower fluorine content and other environmental conditions have been beneficial and responsible for good teeth preservation. One of these areas is being studied by dental investigators and has been recently reported by Taylor.[43] Cheyne[44] also reports cases of beneficial fluorine treatments of children for a period of one year, and notes twice as many cases of dental caries in the control group as in the fluorine-treated group.

5. Aluminum

It has not been proved that aluminum is an essential element of body tissue, but all plants and animals contain appreciable quantities of this element. The average human diet contains about 3.0 g. per week, part of which comes from the use of aluminum cooking utensils, the rest from food. There is no evidence to indicate that a normal amount of aluminum in the diet is detrimental. McCollum and associates[45] report no difference in growth and reproduction of rats when fed either a ration containing 0.6% aluminum or an aluminum-free ration. When excess aluminum salts have been added to the diet of rats, there is a development of rickets, due to the interference in phosphorus absorption. There is evidence to indicate that the element is detrimental to the corn plant. As reported by Hoffer and Carr,[46] aluminum and iron accumulate in the nodes of the plant, preventing the normal movement of the sap throughout the plant when grown in acid soil (Fig. 16, see page 275). Nearly 57% of the ash of lycopodium is aluminum, but the ash of most plants contains aluminum only in small amounts.

---

[42] H. T. Dean, *J. Am. Med. Assoc.*, **107**, 1269 (1936).

[43] Ed. Taylor, *Science*, **94**, 2445 (1941).

[44] V. P. Cheyne, *J. Am. Dental Assoc.*, **29**, 804 (1924).

[45] E. V. McCollum, O. S. Rask, and J. E. Becker, *J. Biol. Chem.*, **77**, 754 (1928).

[46] G. N. Hoffer and R. H. Carr, *J. Agr. Research*, **23**, 10 (1923).

6. Arsenic

Another trace element, arsenic, is widely distributed in the human body, especially in the nails and hair, where it is present to the extent of 0.10 mg. per 100 g. It is known that the use of arsenic compounds dates back to the middle ages, when it was used by the Styrians to clear complexions and increase weight. To avoid toxicity, arsenic must be taken in very small doses at first and increased gradually until a tolerance has been acquired. It is often taken internally in the form of Fowler's solution (1% Na_3AsO_3) for skin diseases, tonic, syphilis, and for sleeping sickness. Arsenic is found "normally" in many persons, but it has not been proved to be an essential element. Forbes and associates[47] have shown in a study of metabolism of the milch cow, that arsenic is not normally excreted in the milk unless given in excess. When shrimp containing arsenic was fed to rats, it was noted that only a slight amount of arsenic was stored; however, when inorganic arsenic compounds were fed to the rats it was stored in large quantities. This result indicates that storage in the animal body depends in part on the form in which arsenic is administered.

7. Silicon

Silica is present in many plant and animal foods. Timothy hay may contain as high as 1.4% silica, while an animal product like the ash of feathers may contain as much as 77%. Silicon is not known to be essential to the life of a plant or animal, although it occurs in all tissues and is present in the newborn to the extent of 0.05 g. SiO_2. It may be of more importance in the development of plant life than in that of animals, as it appears to be a part of the leaf structure and forms a gel material of chemical importance in keeping a water balance in the plant. When the silicic acid gel, H_2SiO_3, in corn leaves, for example, dries out leaving the SiO_2 residue in the edge of the leaf, it forms a rough cutting blade familiar to all who have worked with the dried fodder.

8. Selenium

Franke,[48] of South Dakota, determined the cause of an old and baffling disease of livestock known locally as "alkali disease" or "blind staggers." He found that the trouble was caused by the presence of selenium in the soil and vegetation. Soils that contain only 9 p. p. m. of selenium may grow plants that contain as much as 1200 p. p. m. of the element. It requires only 500 to 800 p. p. m. in vegetation to produce an acute poisoning in the cattle. The disease manifests itself in horses by the loss of hair in the mane and tail and by abnormal hoof development, in addition to the sloughing off of hoofs already formed. The liver also is seriously deformed. The animals lose the desire for food and in some cases starve to death. The milk and eggs from cows and hens fed selenium rations contain this element in some degree but they are not considered a serious menace to public health. A study of plants made poisonous by selenium has been reported by Trelease and Martin.[49] There has been no method developed to counteract the poisonous effect of selenium in either plants or soil; hence the abandonment of the infected area for livestock appears to be necessary for the present.

---

[47] E. F. Forbes, J. A. Schultz, C. H. Hunt, and C. H. Winter, *J. Biol. Chem.*, **52**, 281 (1922).

[48] K. W. Franke, *J. Nutrition*, **8**, 597 (1934).

9. Cobalt and Nickel

The most recent of the elements to secure a place in nutritional studies are cobalt and nickel. A fatal disease of sheep and cattle in Australia and New Zealand called "pine disease" has been found by Underwood[50] to be connected with a deficiency of these elements in the soil and may be cured by the addition of small amounts of cobalt added to the salt supply. Nickel added in traces to the cobalt increased the beneficial action of the cobalt salt to the sheep according to Dixon,[51] of New Zealand (Fig. 20). The symptoms of cobalt deficiency include loss of appetite, pallor, and emaciation of the body. There is usually a reduction of the red blood cell count as well as of the hemoglobin content. Only traces of cobalt are needed in the diet and these are usually supplied from the soil and are found in most vegetation. As little as 1 mg. of cobalt supplied daily to the affected sheep for a period of two weeks will effect a cure. It has not been shown that nickel is an essential element in nutrition, but cobalt, it appears, must be considered essential. The presence of cobalt and nickel in the pancreas was first noted in 1926 by Bertraud and Macheboeuf.[52] It has been found that the addition of cobalt to a normal diet of rats produces polycythemia, the cells being greatly increased in number, but it does not cure anemia in rats.

10. Bromine

Bromine is a normal constituent of all plant and animal tissues, but until recently little attention had been given to the physiological importance of the element. Its action in the body is similar to that of chlorine, but the two are not interchangeable. Blood serum is known to contain about 1 mg. of bromine per 100 ml., whereas the cells contain about 0.5 mg. per 100 ml. The bromine content of plants varies from 0.18 to 2.0 mg. per 100 g. of dry material, with watermelon and tomatoes having the largest amount. Winnek and Smith[53] have experimented with a synthetic rat diet containing only 0.5 mg. of bromine per kg. and noted that the rats grew from weaning to maturity and even produced young, though the young did not survive. It is a difficult matter to determine the bromine requirement of the animal body as such minute traces of this element appear to be required.

---

[49] S. F. Trelease and A. L. Martin, *Botan. Rev.*, **2**, 373 (1936).

[50] E. J. Underwood, *Proc. Soc. Exptl. Biol. Med.*, **36**, 296 (1937).

[51] J. K. Dixon, *New Zealand J. Sci. Tech.*, **18**, 326 (1937).

[52] G. Bertraud and M. Macheboeuf, *Compt. rend.*, **182**, 1305 (1926).

[53] P. S. Winnek and A. H. Smith, *J. Biol. Chem.*, **121**, 345 (1937).

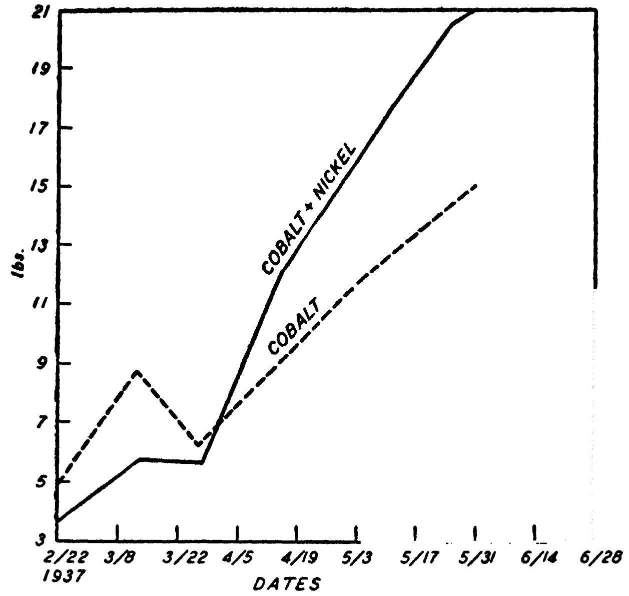


Fig. 20.—Average increase per lamb from initial weights. Experiment started February 2, 1937.[51]

11. Boron

The element boron has been shown by McHargue and Calfee[54] to be essential for the growth of lettuce and other plants. The exact role of this element is not known, but its absence affects the meristematic tissues, causing blackening and finally death of the plant. Boron is toxic to plants, however, when present in appreciable concentration causing the plant to develop chlorosis. Boron in toxic quantities was formerly present in potash fertilizers derived from potash minerals in the western part of the United States, but this element was later largely eliminated from fertilizers. One pound of anhydrous borax ($Na_2B_4O_7$) per acre is slightly toxic to the corn plant although 4 lbs. per acre has been found beneficial for the growth of oats.

IX. GROUP EFFECTS OF MINERALS IN THE ANIMAL BODY

In addition to the effect of the different elements in the body and their definite relationships to each other, there are also certain physiological effects resulting from such group intakes as occur when mineral waters are consumed. Besides there are the effects of accumulation of certain acid and base ions which have to be considered if the neutrality of the body is to be maintained.

---

[54] J. S. McHargue and R. K. Calfee, *Plant Physiol.*, **8**, 305 (1933).

1. Alkaline and Salt Waters

In many parts of the world the natural water supplies are loaded with salts of such high concentration as to make them questionable for drinking purposes. Heller,[55] of Oklahoma, has made a study of the tolerance of animals to certain salts and to high concentrations of minerals in the body. He found that the injury depended on the total amount of salts dissolved in the water rather than on any specific compound. This result indicates that damage is caused by the osmotic action rather than the ion effect. The limit of concentration tolerated was found to be about 1.7%, and alkaline waters were found to be more harmful to the animal than neutral salts. It was noted, also, that magnesium chloride was more harmful than calcium or sodium salts, and that chlorides were generally less injurious than sulfates. Sheep proved to be less affected by salts in the drinking water than cattle, while hogs were the most sensitive to its action.

2. Acid-Base Balance in the Diet

Much study has been given to the question of the influence of the acid-base balance of the diet on metabolism. It is known that the *p*H of the blood and body fluids does not vary widely from normal in health, but it has not been demonstrated that the alkaline or acid-ash value of a ration is either harmful or beneficial. Noorden[56] has aptly summarized the data: "Everywhere, whether from north or south, east or west, from whatever countries, races, or sects, whether the food comes from acidifying meat, eggs, cheese, cereal, and legume diets or from alkalizing milk, fruit, root and leafy vegetable diets the same actual *p*H of blood occurs." The urine of carnivora is commonly acid, that of herbivora is usually alkaline, while that of omnivora is either alkaline or acid depending on the diet. Cattle can use large quantities of lactic and acetic acids, as well as other organic acids, present in silage without unbalancing seriously the *p*H of the blood. Even the mineral acids used in preserving silage by the A. I. V. process have caused no known detrimental effects to cattle. The organic acids of foods are readily oxidized in the animal body, while the mineral acids may be neutralized by the production of ammonia from urea. The acid-forming mineral elements of the body are phosphorus, chlorine, and sulfur; the base-forming elements are calcium, magnesium, sodium, and potassium. The acid-base balance of food is determined by calculating the equivalent in acid and base in terms of normal solutions. Thus a cereal such as wheat is called an acid-forming food because the ash acidity is greater than the alkalinity by 12 ml. of normal acid per 100 g. Roughages like clover hay are base forming, as the hay has a potential alkalinity of 86 ml. The bones of the body are the main reserve of base-forming or positive minerals. It has been calculated that the average total ash of the skeleton weighs about 2250 g. with a base equivalent of 30 ml., which is relatively an enormous store of positive minerals. The calculation of the bone equivalent is made on the basis of its being composed of 84% trivalent calcium phosphate, $Ca_3(PO_4)_2$, and 13% calcium carbonate, $CaCO_3$. The valency of phosphate salts in the body fluids is equivalent to 1.8; when the salt is excreted in acid urine, it has a valency of 1.0. Thus PO_4^{--}, when converted to $H_2PO_4^-$, requires two less equivalents to neutralize the acid, or $Ca_3(PO_4)_2$ has two moles of phosphate. One gram of bone salts furnishes a total of 13.4 equivalents of cations made up of 10.8 from calcium phosphate and 2.6 from calcium carbonate.

---

[55] V. G. Heller, *J. Nutrition*, **5**, 421 (1932). V. G. Heller, Oklahoma Agr. Expt. Sta., *Bull.* **217** (1933).

[56] C. Noorden, *Alte und neuzeitliche Ernährungsfragen.* Springer, Berlin, 1931.

TABLE 30

POSITIVE AND NEGATIVE ION COMPOSITION OF SEA WATER

	Na^+	K^+	Ca^{++}	$Mg.^{++}$	Cl^-	CO_3^{--}	SO_4^{--}	Total positive	Total negative
G./kg.	11.0	0.4	0.4	1.3	19.0	0.09	2.7	...	...
M eq./kg.	480.0	10.0	20.0	108.0	535.0	18.00	56.0	618	609
								Positive excess eq. 9.0	

An alkaline-ash diet is considered most desirable by many students of nutrition, notably Berg[57] in Germany and Bischoff and associates[58] in the United States. They contend that since the body usually excretes an excess of mineral anions in the urine it would cause a strain on the excretion mechanism to handle an acid ash. Again, the alkaline-ash advocates contend that because milk is provided by nature and is an alkaline-ash food it represents the ideal condition.

TABLE 31

COMPARISON OF TOTAL POSITIVE AND NEGATIVE EQUIVALENTS IN BODY FLUIDS

Positive eq.	Na 2.8	K 3.5	Ca 57.3	Mg. 1.7	Total 65.3
Negative eq.	P 58.0	Cl 2.4	S ...	HCO_3 ...	Total 60.4
				Positive excess 4.9	

Macallum[59] has called attention to the mineral composition of sea water and has made a comparison between the total positive and negative ions contained in sea water and in blood serum. The mineral composition of sea water is noted in Table 30.

A similar comparison of the total positive and negative equivalents of body fluids by Shohl[60] is given in Table 31. It will be noted from this table that the calcium is the big positive factor and phosphorus the important negative element concerned with body acid-base equilibrium.

---

[57] R. Berg, *Controlle des Mineralstoffwechsels.* Hirzel, Leipzig, 1930.

[58] F. Bischoff, W. D. Sanaum, M. L. Long, and M. M. Dewar, *J. Nutrition*, **7**, 51 (1934).

[59] A. B. Macallum, *Physiol. Revs.*, **6**, 316 (1926).

[60] A. T. Shohl, *Mineral Metabolism.* Reinhold, New York, 1939, p. 36.

X. SOIL FACTORS DETERMINE NUTRITIONAL QUALITY OF FOOD

It has been the custom of many food investigators to take for granted that the mineral content of such products as potatoes, cabbage, celery, grasses, etc., is approximately constant in mineral composition with little appreciation of any variability as determined by soil and cultural factors. There are large areas in different countries where losses have occurred because of low phosphorus content. The Tennessee State Experiment Station reported a marked difference in the phosphorus content of lespedeza grown on Maury silt loam in a good phosphoric limestone basin soil, and that grown on Clarksville poor silt loam soil low in phosphorus. The former contained 0.27% phosphorus in contrast to the latter which contained only 0.10% phosphorus. Williams and associates[61] have studied, with the use of rats, the nutritive value of lespedeza, alfalfa, soybeans, and red clover hays grown on the good and poor soils mentioned above. They noted that when the high and low phosphorus hays of the same kind, in amounts which furnished the same phosphorus intakes, were added to diets otherwise adequate the high phosphorus hay proved much superior. It is believed the difference was due to differences in digestibility rather than in the forms of phosphorus. The Massachusetts Agricultural Experiment Station[62] has made a study of minerals in nutrition and has come to the conclusion that many of the common food products may not be as nutritionally adequate as has been generally assumed. They have attempted to get factual evidence; among other studies they have tried to grow crops with the added cations—sodium, potassium, magnesium, and calcium, and with the added anions—chlorides, sulfates, and phosphates. It was found possible to increase the mineral content of some kinds of plants, depending on the crop, the mineral, and the other chemical substances present. The calcium intake of the plants, cabbage, celery, and lettuce, was decreased when sodium and calcium salts were added together. Potassium also was found to decrease the calcium intake, and to increase the potassium content of the plants. When cabbage plants were treated with both sodium and potassium salts the potassium intake was greatly increased. It is suggested by these investigations that the time may come when the necessary minerals may be controlled by the kind of soil and the cultural methods used to grow vegetables.

XI. VITAMIN AND MINERAL FORTIFICATION OF FOOD

May 27, 1940, is a date worth noting in food history, as it was the first day that fortified or "enriched" bread became generally available throughout the United States, coincident with the National Nutrition Conference called by President Roosevelt. The specifications for enriched bread as noted in the *Federal Register* of that date require that the bread shall contain not less than 1 or more than 2 mg. of thiamine (vitamin B_1) per pound of baked bread; also, not less than 4 or more than 8 mg. of nicotinic acid (or nicotinamide), and not less than 4 or more than 16 mg. of iron per pound. As optional ingredients enriched bread may also contain not less than

---

[61] D. E. Williams, F. L. Macleod, and E. J. Morrell, *J. Nutrition*, **19**, 251 (1940); **20**, 391 (1940).

[62] "Minerals in Nutrition," Dept. of Agronomy, Mass. Agr. Expt. Sta., *Bull.* **374** (1940).

0.8 or more than 1.6 mg. of riboflavin (vitamin B_2), not less than 150 or more than 600 U. S. P. units of vitamin D, and not less than 0.3 or more than 1.2 g. of calcium. Riboflavin became a required ingredient of enriched flour in January, 1942. In August, 1942, 65% of all white bread sold was enriched. (See page 711). Lack of public appreciation of the values of enrichment and the highly competitive condition of low-priced flours used by low-income people retarded its acceptance. The public generally refuses to eat 100% whole wheat bread which is rich in vitamins and minerals; hence it was deemed advisable by the committee on Food and Nutrition of the National Research Council to recommend the addition of enriching constituents to white bread. The daily human requirement of thiamine ranges from 1.0 to 2.3 mg. depending on the age, sex, and activity of the consumer. It has been calculated by Toby[63] that twelve 1-ounce slices of bread which has been enriched to the maximum amount would be required to furnish the amount of thiamine contained in one slice of 100% whole wheat bread. The committee finally decided that the amount of thiamine, riboflavin, nicotinic acid, and iron contained in the average wheat was to be the criterion for the proper quantity of restoration or fortification needed without thought of trying to improve on nature by enriching flour above the natural grain content. White bread is often spoken of as a "devitalized" product nearly devoid of vitamins and minerals, but instead it usually contains besides wheat flour about 6% of milk solids with its high calcium and phosphorus content, together with yeast, sugar, salt, and malt extract. Now, to enriched bread is added thiamine, nicotinic acid, riboflavin, and iron. Thus, enriched bread has become more worthy again to be called the "staff of life."

XII. CONCLUSION

It may appear to many people that the addition of a complex mineral mixture and vitamins to food, trying to compete with or improve on nature, is a questionable procedure. This is probably true for all those living out in the open country where they have access to plenty of fresh milk, meat, and vegetables, and where direct sunlight is available to them many of the daylight hours. Those citizens, however, who find it necessary to work by artificial light and who live on packaged and refined food with its uncertain mineral and vitamin content will appreciate the efforts used in the food industry to make their food tasty and nutritious even though it has to be preserved for them in a dried, frozen, canned, or fortified condition. This necessity for enriched food, however, does not justify the many proprietary mineral mixtures now in the market for farm animals which contain potassium, iron, magnesium, sulfur, and copper compounds as well as many other constituents that are sold as "shot gun" prescriptions for tonics, purgatives, or worm remedies.

It is to be hoped that this discussion of minerals in foods will serve to call attention to the special properties of the various elements in their role of supplying the necessary compounds and furnishing a desirable salt balance for both human beings and animals.

[63] J. A. Toby, *Proc. Inst. Food Tech.*, 2nd Conf., 1941.

Scientific feeding has become a necessity for all who hope to continue in the business of raising farm animals; and all scientific nourishment must include, in the proper balance, the necessary minerals.

Selected Bibliography

Bridges, M. A., and Mattice, M. R., *Food and Beverage Analyses.* Lea and Febiger, Philadelphia, 1942.

Bunge, G., *Textbook of Physiological and Pathological Chemistry.* Blakiston, Philadelphia, 1902.

McCollum, E. V., Orent-Keiles, E., and Day, H. G., *The Newer Knowledge of Nutrition.* Macmillan, New York, 1939.

Sherman, H. C., *Chemistry of Food and Nutrition.* Macmillan, New York, 1941.

Sherman, H. C., and Lanford, C. S., *Essentials of Nutrition.* Macmillan, New York, 1943.

Shohl, A. T., *Mineral Metabolism.* Reinhold, New York, 1939

Chapter IX

COLORING MATTERS IN FOODS

C. F. Jablonski

New York, N. Y.

It has been a practice of long standing to resort to coloring in order to improve the appearance of products. This desire for colors has come down from the dawn of history; and present-day excavations yield proof of the knowledge of the art of dyeing possessed by races of remote antiquity. The number of coloring materials has increased from relatively a few simple ones to a vast range of tints and shades mostly of synthetic production, and their use has become an intimate part of our civilization.

Generally speaking, coloring matters can be divided into three major groups: pigments and lakes, coal-tar dyes, and natural coloring matters.

I. PIGMENTS AND LAKES

Pigments are of inorganic constitution, either found as such in nature or prepared synthetically. They do not combine chemically with any fabric, but are applied as a coating by means of adhesives. Many of the common metals in combination with salt-forming groups produce different colored pigments, which, although important in the crafts and arts, are not often used in food products. Pigments which are occasionally encountered in foods are *charcoal,* or other forms of carbon; *ultramarine blue* (containing aluminum, sulfur, and sodium); *Prussian blue* (containing iron); and *talc* (a siliceous mineral). Charcoal can be detected by its indifference toward chemical reagents and by its combustibility. Ultramarine blue is stable toward alkalies, but is decomposed by dilute hydrochloric acid with the liberation of hydrogen sulfide. Prussian blue is decomposed by alkalies, but is unaffected by hydrochloric acid. Talc can be identified by the purple coloration obtained by fusion with cobalt nitrate (test for aluminum). It has been stated in the literature that *chrome yellow* (lead chromate) has been used quite frequently to produce a bright yellow color in candies, confections, etc.; but during the last two decades, it has not been detected in food products.

Unlike the pigments just mentioned, copper sulfate is not insoluble; yet, because of its metallic origin, it is not out of place to refer to it here. Foods such as peas, beans, angelica, plums, cucumbers, edible oils, etc., are sometimes found to be contaminated with copper. This metal has been found present, due perhaps to contact with copper utensils, or to copper compounds having been deliberately added in small amounts to impart a brighter green color to the food. Its presence can be detected by various standard procedures, but preferably by the micromethod of Spacu[1] as copper pyridine thiocyanate.

Lakes are products formed by combining organic coloring matters with metallic salts; they can be prepared from animal or vegetable coloring matters, or from coal-tar dyes. The most important of the animal color lakes is *cochineal carmine.* This is a compound prepared from cochineal and either aluminum or tin. As carmine is very extensively used for coloring food products, it is advisable to ascertain whether the lake contains aluminum, or the more harmful tin, as a base. The reactions for cochineal are given in another paragraph. Lakes produced from vegetable colors are numerous. A few of the most common are the following: *Dutch pink,* a yellow lake, prepared from the extract of quercitron bark and aluminum; *Persian berries,* a yellow lake, prepared from the extract of Persian berries and aluminum; *brazilwood,* a red lake, prepared from the extract of brazilwood and aluminum; *madder,* a red lake, prepared from the extract of madder and aluminum.

Lakes produced from coal-tar dyes have superseded the other types to a great extent. Coal-tar dye lakes may be divided into two groups: (*a*) those produced from *basic dyes* with tannin, tartar emetic, sodium phosphate, or the fatty acid salts of aluminum; and (*b*) those produced from *acid dyes* with salts of lead, barium, zinc, aluminum, tin, antimony, calcium, iron, etc. The color shades obtained are of great variety, but as these lakes seldom enter food products, this subject need not be elaborated.

II. COAL-TAR DYES

Synthetic coal-tar dyes have been known only since the middle of the last century. Previous to that time, coloring matters used for dyeing were of natural origin. Now the great majority of coloring matters are the result of organic synthesis, the products of which in many instances are superior in brilliancy, uniformity, and economy to those obtained from nature. The synthesis of natural coloring matters has brought out the fact that in structure they are, with very few exceptions, derivatives of benzol. In 1856 Perkin accidently discovered a violet dye, mauve. He thus became the first of many pioneers in the field of the coal-tar dye industry. Today over 2000 dyes are known, and many others are constantly appearing, superseding those of less value. The story of coal tar is one of the colorful romances in the history of chemistry.

Selective light absorption by a compound is always associated with the unsaturation of the compound concerned. This selective absorption may be called, in the widest meaning of the word, *color*. When substances absorb light in the ultraviolet or infrared regions, they appear to be colorless. When they absorb light in the visible region, they appear to be colored.

The following are the deductions of Witt[2] concerning the relationships of the structure of dyes and their color:

---

[1] G. Spacu, *Bull. soc. stiinte Cluj,* **1,** 284 (1922).

[2] O. N. Witt, *Ber.,* **148,** 522 (1876).

1. Every dye molecule contains a certain group of atoms which has an especially strong influence in making the substance a dye and it is called a *chromophore* because it is the characteristic coloring group.

2. When the chromophore enters into combination or becomes part of a larger molecule, it is called a *chromogen*. Although chromogens are highly colored substances, they are not dyes.

3. The chromogen is a chemically latent substance, and may be converted to a dye by the addition of some salt-forming group termed an *auxochrome* or color-fixing group. This is usually an amino group (—NH_2, —$NH{\cdot}CH_3$, etc.) to form basic substances, or a hydroxyl (—OH) or a carboxyl (—COOH) group to form acid substances.

According to the atomic theory, the properties of matter depend upon the structure of the atomic nucleus and the number and arrangement of the electrons. Chromophores are groups of atoms with unstable electron configurations. The electrons of these groups of atoms have been transferred to energy-rich quantum states by the absorption of radiant energy.

In general, only those organic compounds that contain either the basic or acid groups can be dyestuffs. Additions of various groups to a dye molecule will produce different shades. The relationship between constitution and color may be illustrated by the following examples: ponceau 2G (aniline + R salt) is an orange dye, and ponceau 3R (cumidine + R salt) is a red dye; also, brilliant orange G (aniline + β-naphtholsulfonic acid) is an orange dye, and fast acid fuchsin (aniline + H acid) is a magenta-red dye. It may be further stated that additions of groups of a higher molecular weight to a dye molecule produce deeper shades; thus, a yellow dye may be changed successively to an orange, red, violet, blue, and green. A further addition to the molecule of the green dye produces again a yellow shade. Yellow is therefore considered a primitive color, and the process of building up the dye molecule is called a positive color change. The reverse (substituting bodies of lower molecular weight) is termed a negative color change.

Coal-tar dyes are finding a diversified usage, and their field of application is by no means exhausted. Besides the dye industry other arts and sciences which are served by these protean sons of soft coal are chemistry (indicators for colorimetric analysis), bacteriology (various stains), manufacture of explosives (salts of picric acid and trinitrotoluene), photography (developers), medicine (antiseptics), and the manufacture of food products. Their use in foods and beverages becomes well-nigh a necessity, particularly in instances where the natural colors of the product have diminished to such an extent as to give them a deceptive appearance of inferior quality.

When coal-tar colors were first manufactured, arsenic acid and mercury were freely employed in the manufacture of fuchsin and other triphenylmethane dyes. Consequently, all coal-tar colors were considered poisonous. Later, when other oxidizing agents displaced arsenic acid, the dyes were assumed to be harmless. Subsequent experiments, however, have proved that a number of coal-tar dyes are by no means harmless even when free from arsenic. Although the literature contains somewhat contradictory statements about the physiologically harmful effects of some dyes, nevertheless there are certain coal-tar colors that produce a decidedly toxic effect upon the human organism. The National Confectioners Association in 1899 issued a list of injurious coloring matters which were considered unfit for coloring food products.

The list contained the following dyes: Biebrich scarlet (280);[3] croceine scarlet (277); new coccine (185); croceine scarlet 8B (286); croceine scarlet O (281); safranine (841); picric acid (7); Martius yellow (9); resorcine yellow (148); Victoria yellow (8); orange II (151); metanil yellow (138); Sudan I (24); orange IV (143); naphthol green B (5); methylene blue (922); Bismarck brown (331); Bismarck brown R (332); fast brown G (175); and chrysoidine (21).

More recent investigations have brought out the fact that many of the above-listed dyes if comparatively pure, *e. g.*, free from intermediates, have proved to be harmless. Some of them are even permitted as colors for food products in foreign countries.

In order to protect the health of the public, the U. S. Government permits food manufacturers to use only such batches of coal-tar dyes as have been tested by the U. S. Food and Drug Administration and have been found by this agency to be harmless and suitable for use. Such dyes are known as "certified" dyes. The procedure for the certification of coal-tar colors is described in the Federal Security Agency, Food and Drug Administration pamphlet, Service and Regulatory Announcements, Food, Drug, and Cosmetics No. 3, *Coal-Tar Color Regulations* issued in September, 1940.

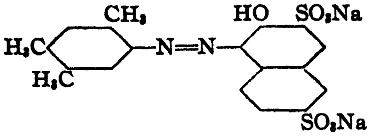
When the Federal Food and Drug Act was first enacted (1907) the number of permitted coal-tar coloring matters certifiable for food purposes was limited to seven. As the need for additional dyes became apparent there was a gradual increase in the number certified for food use. In 1942 eighteen dyes were certifiable. They consist of four oil-soluble dyes and fourteen water-soluble colors, and are officially known as FD&C colors. The designation FD&C indicates that they may be used in food, drugs, and cosmetics. They are subdivided into FD&C Reds, FD&C Oranges, FD&C Yellows, FD&C Greens, and FD&C Blues. The complete list follows:

FD&C Colors	No.	Former Name	Colour Index No.
Red	1	Ponceau 3R	80
Red	2	Amaranth	184
Red	3	Erythrosin B	773
Red	4	Ponceau SX	...
Red	32	Oil Red XO	..
Orange	1	Orange I	150
Orange	2	Orange SS	...
Yellow	1	Naphthol Yellow S, sodium salt	10
Yellow	2	Naphthol Yellow S, potassium salt	10
Yellow	3	Yellow AB	22
Yellow	4	Yellow OB	61
Yellow	5	Tartrazine	640
Yellow	6	Sunset Yellow FCF	...
Green	1	Guinea Green B	666
Green	2	Light Green SF Yellowish	670
Green	3	Fast Green FCF	...
Blue	1	Brilliant Blue FCF	...
Blue	2	Indigotin (Indigo Carmine)	1180

---

[3] The numbers in parentheses correspond to the Colour Index Classification.

1. FD&C Red No. 1



Ponceau 3R ($C_{19}H_{16}N_2O_7S_2Na_2$) is the sodium salt of pseudocumylazo-β-naphthol-3,6-disulfonic acid. This dye belongs to the monazo group of dyes and is the product formed by diazotizing one mole of pseudocumidine and coupling it with one mole of β-naphthol-3,6-disulfonic acid (R salt). Ponceau 3R is a dark red powder which dissolves readily in water, giving a cherry-red solution; it is also slightly soluble in 95% alcohol.

Reactions of Ponceau 3R

Aqueous solution of the dye and $^1/_{10}$ volume of concentrated hydrochloric acid—trifle redder.

Aqueous solution of the dye and $^1/_{10}$ volume of concentrated ammonium hydroxide—yellow.

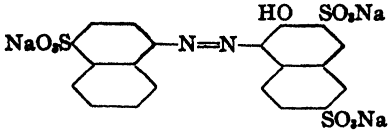
To 10 ml. of approximately 0.05% solution of the dye add 1 ml. of concentrated hydrochloric acid, 2 ml. of saturated bromine water, and an excess of a saturated solution of hydrazine sulfate. Couple immediately with a solution containing two drops of 1% alcoholic α-naphthol in 10 ml. of 2 *N* sodium carbonate to obtain a light pink solution, which when extracted by ether gives a light yellow solution. This ethereal solution, after being washed twice with small portions of water, produces a purplish coloration upon addition of 2 volumes of concentrated hydrochloric acid.

A neutral aqueous solution of the dye is readily decolorized by sodium hyposulfite, while an alkaline solution is decolorized only with great difficulty. A very sensitive test for ponceau 3R is the immediate formation of a bluish-red precipitate upon the addition of a solution of barium chloride to a neutral aqueous solution of the dye. Ponceau 3R is almost completely extracted from *N*/16 hydrochloric acid by amyl alcohol, from which it may be removed with 5% salt solution. The subsidiary dyes are removed (after all the ponceau 3R has been extracted with 5% salt solution) by diluting the amyl alcohol solvent with equal portions of petroleum benzine, extracting with water, and estimating with standard titanium trichloride.

Spot Tests on Dyed Wool.—

Concentrated hydrochloric acid—rose-red.
Concentrated sulfuric acid—scarlet.
10% Sodium hydroxide—orange.
Concentrated ammonium hydroxide—no change.

2. FD&C Red No. 2



Amaranth ($C_{20}H_{11}N_2O_{10}S_3Na_3$) is the sodium salt of 4-sulfo-α-naphthylazo-β-naphthol-3,6-disulfonic acid. This dye belongs to the monazo group of dyes and is the product formed by diazotizing one mole of naphthionic acid and coupling it with one mole of β-naphthol-3,6-disulfonic acid (R salt). Amaranth is a reddish-brown powder readily soluble in water, giving a magenta-red solution; it is also sparingly soluble in 95% alcohol.

Reactions of Amaranth

Aqueous solution of the dye and $^1/_{10}$ volume of concentrated hydrochloric acid—somewhat brighter.

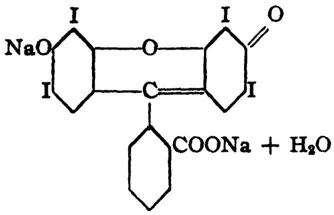
Aqueous solution of the dye and $^1/_{10}$ volume of concentrated ammonium hydroxide—browner.

To 10 ml. of approximately 0.05% solution add 1 ml. of concentrated hydrochloric acid, 2 ml. of saturated bromine water, and an excess of a saturated solution of hydrazine sulfate, and couple immediately with a solution containing 2 drops of 1% alcoholic α-naphthol in 10 ml. of 2 *N* sodium carbonate. A purplish-red solution of fast brown N (175) is obtained. A neutral or alkaline solution of amaranth is readily decolorized by sodium hyposulfite, while an acid solution is decolorized with difficulty. Amaranth is almost completely extracted from a 4 *N* hydrochloric acid solution by amyl alcohol, from which it may be removed by 0.25 *N* hydrochloric acid. The isomeric and lower sulfonated dyes that may be present in amaranth are new coccine (185) and fast red E (182). The latter is estimated by first removing the amaranth with 0.25 *N* hydrochloric acid and then employing the method used for separating fast wool yellow from tartrazine. New coccine may be estimated by precipitating the amaranth with 0.1 *N* benzidine solution, filtering, and estimating the filtrate colorimetrically. The cyanide test may also be used to differentiate between amaranth and new coccine. This is based upon the fact that the sulfo group in the 3-position is replaced by cyanogen. Heat for 5 min. in a boiling water bath 10 ml. of the neutral solution, to which 1 ml. of 20% ammonium chloride solution and 1 ml. of 20% potassium cyanide solution have been added; no perceptible change is noted with new coccine, but amaranth becomes brownish. The solubility ratio of new coccine after this treatment is not changed, but that of amaranth is altered.

Spot Tests on Dyed Wool.—

Concentrated hydrochloric acid—darker.
Concentrated sulfuric acid—violet.
10% Sodium hydroxide—dull brownish.
Concentrated ammonium hydroxide—no change.

3. FD&C Red No. 3



Erythrosin ($C_{20}H_6O_5I_4Na_2 \cdot H_2O$) is the sodium salt of tetraiodofluorescein. It belongs to the xanthene group of dyes and is the product formed by iodinating fluorescein (a dye made by condensing phthalic anhydride with resorcinol). It is a brown powder, easily soluble in 95% alcohol, giving a red solution and slight fluorescence.

Reactions of Erythrosin B

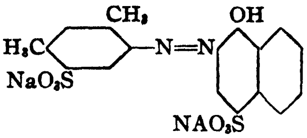
An aqueous solution of the dye upon addition of $^1/_{10}$ volume of concentrated hydrochloric acid becomes yellow with the formation of a reddish-orange precipitate of the color acid. However, in an alcoholic solution of the dye, hydrochloric acid will not precipitate the color acid.

An aqueous solution of the dye and $^1/_{10}$ volume of concentrated ammonium hydroxide produces no perceptible color change. Erythrosin B is almost completely extracted from a 5% salt solution by amyl alcohol; also from *N* hydrochloric acid solution by ether, from which it may be removed by the addition of weak alkalies; or from amyl alcohol by diluting with petroleum benzine and extracting with weak alkalies. The most important organic subsidiary dye that may be present in erythrosin B is the diiodofluorescein compound (erythrosin G). A fairly accurate method for estimating this impurity is as follows: Extract a 0.1 *N* hydrochloric acid solution of the dye with several portions of benzol. (The solvent will contain almost all the erythrosin G, and some erythrosin B.) Extract the combined benzol layers with several portions of concentrated hydrochloric acid until the acid solution is colorless; partly neutralize the combined acid extracts and shake out with ether. The ether extract will contain the erythrosin G in a fairly pure state.

Spot Tests on Dyed Wool.—

Concentrated hydrochloric acid—orange-yellow.
Concentrated sulfuric acid—orange-yellow.
10% Sodium hydroxide—no change.
Concentrated ammonium hydroxide—no change.

4. FD&C Red No. 4



Ponceau SX ($C_{18}H_{14}N_2O_7S_2Na_2$) is the sodium salt of 5-sulfo-*m*-xylylazo-α-naphthol-4-sulfonic acid. It belongs to the monazo group of dyes and is the product formed by diazotizing one mole of 1-amino-2,4-dimethylbenzene-5-sulfonic acid and coupling it with one mole of naphthionic acid. It is a red powder easily soluble in water, giving an orange-red solution, and it is slightly soluble in 95% alcohol.

Reactions of Ponceau SX

Aqueous solution of the dye and $^1/_{10}$ volume of concentrated hydrochloric acid—no perceptible change.

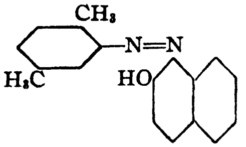
Aqueous solution of the dye and $^1/_{10}$ volume of concentrated ammonium hydroxide—orange.

To 10 ml. of approximately 0.05% solution of the dye add 1 ml. of concentrated hydrochloric acid, 2 ml. of saturated bromine water, and an excess of a saturated solution of hydrazine sulfate, and couple immediately with a solution containing two drops of 1% alcoholic α-naphthol in 10 ml. of 2 *N* sodium carbonate. A yellowish-red solution is obtained which is not soluble in ether (differing from ponceau 3R). A neutral or alkaline solution of the dye is decolorized by sodium hyposulfite, while an acid solution is decolorized with difficulty. Barium chloride solution added to an aqueous solution of the dye does not produce a precipitate immediately (differing from ponceau 3R), but on prolonged standing an orange-red precipitate is deposited. Ponceau SX is almost completely extracted from a *N*/16 hydrochloric acid solution by amyl alcohol, from which it may be removed by 5% salt solution. To obtain the lower sulfonated colors, first remove ponceau SX with 5% salt solution until no more dye is extracted, then dilute the amyl alcohol extract with an equal volume of petroleum benzine, remove the dye with several portions of water, and titrate with standard titanium trichloride.

Spot Tests on Dyed Wool.—

Concentrated hydrochloric acid—trifle deeper red.
Concentrated sulfuric acid—deeper red.
10% Sodium hydroxide—orange-yellow.
Concentrated ammonium hydroxide—orange-yellow.

5. FD&C Red No. 32



Oil red XO ($C_{18}H_{16}N_2O$), *m*-xylylazo-β-naphthol, belongs to the monazo group of dyes and is the product obtained by diazotizing one mole of xylidine mixture (from which the meta component has been partially removed) with one mole of β-naphthol. Oil red XO is a brownish-red powder insoluble in water but soluble in alcohol, giving an orange-red color.

Reaction of Oil Red XO

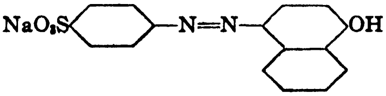
Alcoholic solution of dye and $^1/_{10}$ volume of concentrated hydrochloric acid—slightly redder.

Alcoholic solution of dye and $^1/_{10}$ volume of concentrated ammonium hydroxide—no change.

Spot Tests on Dyed Silk.—

Concentrated hydrochloric acid—bright red.
Concentrated sulfuric acid—bright red.
10% Sodium hydroxide—orange.
Concentrated ammonium hydroxide—orange.

6. FD&C Orange No. 1



Orange I ($C_{16}H_{11}N_2O_4SNa$) is the sodium salt of *p*-sulfophenylazo-α-naphthol. This dye belongs to the monazo group of dyes and is the product formed by diazotizing one mole of *p*-sulfanilic acid and coupling it with one mole of α-naphthol. The product is a reddish-brown powder easily soluble in water, giving an orange-red solution; it is also soluble to a considerable extent in 95% alcohol.

Reactions of Orange I

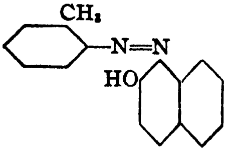
Aqueous solution of the dye and $^1/_{10}$ volume of concentrated hydrochloric acid—slightly redder; upon further addition of acid, a purplish-red color is produced.

Aqueous solution of the dye and $^1/_{10}$ volume of concentrated ammonium hydroxide—red.

When orange I is treated with bromine and saturated hydrazine sulfate and coupled with α-naphthol, as described under FD&C Red No. 1, and the resulting orange-red colored solution is extracted with ether, no color should be imparted to the ether upon addition of 2 volumes of concentrated hydrochloric acid (absence of croceine orange (26)). A neutral or acid solution of orange I is readily decolorized by sodium hyposulfite, while an alkaline solution is decolorized with difficulty. Orange I in 5% salt solution is almost completely extracted by amyl alcohol, from which it may be removed by *N* sodium carbonate solution. Orange I may be contaminated with the objectionable orange II. In order to estimate the latter, wash out the amyl alcohol extract with equal portions of *N* sodium carbonate solution until no more color is extracted; dilute the solvent with equal portions of petroleum benzine, extract the orange II with water, and estimate with standard titanium trichloride.

Spot Tests on Dyed Wool.—
Concentrated hydrochloric acid—bluish red.
Concentrated sulfuric acid—purple.
10% Sodium hydroxide—red.
Concentrated ammonium hydroxide—red.

7. FD&C Orange No. 2



Orange SS ($C_{17}H_{14}N_2O$) *o*-tolylazo-β-naphthol, belongs to the monazo type of dyes and is the product obtained by diazotizing one mole of *o*-toluidine and coupling it with one mole of β-naphthol. Orange SS is an orange powder insoluble in water but easily soluble in 95% alcohol, giving an orange color.

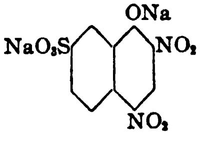
Reactions of Orange SS

Alcoholic solution of dye and ¹/₁₀ volume of concentrated hydrochloric acid—slightly redder.

Alcoholic solution of dye and ¹/₁₀ volume of concentrated ammonium hydroxide—no change.

Spot Tests on Dyed Silk.—
Concentrated hydrochloric acid—redder.
Concentrated sulfuric acid—redder.
10% Sodium hydroxide—orange-yellow.
Concentrated ammonium hydroxide—no change.

8. FD&C Yellow No. 1



Naphthol yellow S ($C_{10}H_4N_2O_8SNa_2$) is the sodium salt of 2,4-dinitro-α-naphthol-7-sulfonic acid and belongs to the nitro group of coloring matters. It is prepared by the nitration of tri- or disulfonic acids of α-naphthol or from the nitroso compound of the 2,7-disulfonic acid. The sodium salt of naphthol yellow S is a light yellow or orange-yellow powder, and, being a nitro compound, burns readily, accompanied by scintillation. It is easily soluble in water, giving a bright yellow solution, but only slightly soluble in 95% alcohol.

Reactions of Naphthol Yellow S

Aqueous solution of dye and $^1/_{10}$ volume of concentrated hydrochloric acid—almost colorless.

Aqueous solution of dye and $^1/_{10}$ volume of concentrated ammonium hydroxide—no change.

Aqueous solution of dye and sodium hyposulfite—red, then colorless.

Aqueous solution of dye and stannous chloride solution and excess of potassium hydroxide solution—pink.

The coloring matter from a solution of naphthol yellow S in *N*/16 hydrochloric acid is almost completely extracted by amyl alcohol, from which the dye may be removed with water. The most deleterious organic impurity of naphthol yellow S is Martius yellow (9). To prove its presence and estimate its quantity proceed as follows: To 5.0 g. of dye in 150 ml. of water add 5 ml. of concentrated hydrochloric acid, and then shake the mixture vigorously with 50 ml. of petroleum benzine (sp. gr. 0.65). After separation, draw off the lower layer and extract it again with 30 ml. of the solvent. Combine the solvent extracts and wash them with 25-ml. portions of 0.25 *N* hydrochloric acid until the washings are colorless. Extract the petroleum benzine with a few 5-ml. portions of 5% sodium hydroxide solution to remove the Martius yellow. Neutralize the alkaline extract with tartaric acid and titrate with standard titanium trichloride, or estimate colorimetrically in neutral solution by comparison with standard naphthol yellow S solution, the tinctorial value of which is considered 80% of that of Martius yellow.

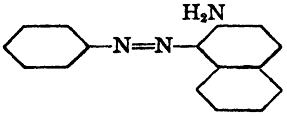
Spot Tests on Dyed Wool.—

Concentrated hydrochloric acid—nearly decolorized.
Concentrated sulfuric acid—paler.
10% Sodium hydroxide—no change.
Concentrated ammonium hydroxide—no change.

9. FD&C Yellow No. 2

Naphthol yellow S (potassium salt) ($C_{10}H_4N_2O_8SK_2$) is prepared in similar manner as the FD&C yellow No. 1, but potassium is substituted for sodium. The reactions with reagents as well as spot test are similar to the sodium dye.

10. FD&C Yellow No. 3



Yellow AB ($C_{16}H_{13}N_3$), phenylazo-β-naphthylamine, belongs to the monazo group of dyes, and is the product obtained by diazotizing one mole of aniline and coupling it with one mole of β-naphthylamine. Yellow AB is an orange powder insoluble in water but easily soluble in 95% alcohol, giving a yellow color.

Reactions of Yellow AB

Alcoholic solution of dye and $^1/_{10}$ volume of concentrated hydrochloric acid—red color.

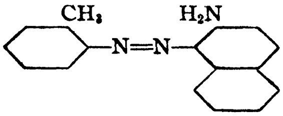
Alcoholic solution of dye and $^1/_{10}$ volume of concentrated ammonium hydroxide—no change.

A petroleum benzine solution of the dye and an equal volume of a solution containing one part of formaldehyde and four parts of acetic anhydride will produce a red coloration in the lower layer.

Spot Tests on Dyed Silk.—

Concentrated hydrochloric acid—orange-red.
Concentrated sulfuric acid—bluish red.
10% Sodium hydroxide—no change.
Concentrated ammonium hydroxide—no change.

11. FD&C Yellow No. 4



Yellow OB ($C_{17}H_{15}N_3$), *o*-tolylazo-*β*-naphthylamine, belongs to the monazo group of dyes and is the product obtained by diazotizing one mole of *o*-toluidine and coupling it with one mole of *β*-naphthylamine. Yellow OB is an orange powder insoluble in water but easily soluble in 95% alcohol, giving a yellow color.

Reactions of Yellow OB

Alcoholic solution of the dye and $^1/_{10}$ volume of concentrated hydrochloric acid—red color.

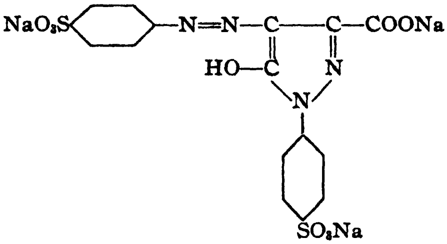
Alcoholic solution of the dye and $^1/_{10}$ volume of concentrated ammonium hydroxide—no change.

A petroleum benzine solution of the dye and an equal volume of a solution containing one part of formaldehyde and four parts of acetic anhydride will produce an orange coloration in the lower layer (differing from yellow AB).

Spot Tests on Dyed Silk.—

Concentrated hydrochloric acid—orange-red.
Concentrated sulfuric acid—bluish red.
10% Sodium hydroxide—no change.
Concentrated ammonium hydroxide—no change.

12. FD&C Yellow No. 5



Tartrazine ($C_{16}H_9N_4O_9S_2Na_3$) is the sodium salt of 4-*p*-sulfophenylazo-1-*p*-sulfophenyl-5-hydroxypyrazole-3-carboxylic acid, and belongs to the pyrazolone group of dyes. The principal method of preparation consists in treating two moles of phenylhydrazine-*p*-sulfonic acid with one mole of dioxytartaric acid. Tartrazine is an orange-yellow powder, easily soluble in water, giving a golden-yellow solution; it is also slightly soluble in 95% alcohol.

Reactions of Tartrazine

Aqueous solution of dye and $^1/_{10}$ vol. of concentrated HCl—no change.

Aqueous solution of dye and $^1/_{10}$ vol. of concentrated NH_4OH—slightly intensified.

For a convenient test for tartrazine, proceed as follows: To 10 ml. of approximately 0.05% solution of the dye add 1 ml. of concentrated hydrochloric acid and 2 ml. of saturated bromine water, and then add an excess of a saturated solution of hydrazine sulfate; couple immediately with a solution containing two drops of 1% alcoholic α-naphthol in 10 ml. of 2 *N* sodium carbonate solution. In the presence of tartrazine, the cherry-red color of orange I will be produced. (This reaction is not specific for tartrazine alone, but occurs whenever *p*-sulfanilic acid is coupled with α-naphthol.)

This alkaline solution should not yield any color to ether (absence of orange G (27)). An aqueous solution of tartrazine, acidified with acetic acid, reduced with zinc dust, and then filtered, will, upon standing, develop a purplish coloration. Tartrazine is not readily decolorized by sodium hyposulfite, the alkaline solution being more resistant. Tartrazine in 4 *N* hydrochloric acid solution is almost completely extracted by amyl alcohol, from which it may be removed by 0.25 *N* hydrochloric acid. Fast wool yellow (637) is the lower sulfonated dye of tartrazine, and may be estimated as follows: Remove tartrazine by washing the amyl alcohol extract with equal volumes of 0.25 *N* hydrochloric acid until no more color is extracted; dilute the amyl alcohol with an equal volume of petroleum benzine; remove the dye with water, and estimate the dye with standard titanium trichloride.

Spot Tests on Dyed Wool.—

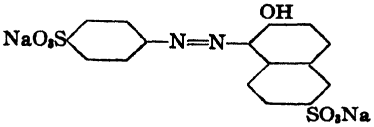
Concentrated hydrochloric acid—slightly darker.

Concentrated sulfuric acid—darker.

10% Sodium hydroxide—no change.

Concentrated ammonium hydroxide—no change.

13. FD&C Yellow No. 6



Sunset yellow FCF ($C_{16}H_{10}N_2O_7S_2Na_2$) is the sodium salt of *p*-sulfophenylazo-β-naphthol-6-monosulfonate. The dye belongs to the monazo group of dyes and is the product obtained by diazotizing one mole of *p*-sulfanilic acid and coupling it with one mole of β-naphthol-6-sulfonic acid (Schaeffer's acid). Sunset yellow FCF is an orange powder, easily soluble in water, giving an orange-yellow solution. It is only slightly soluble in 95% alcohol.

Reactions of Sunset Yellow FCF

Aqueous solution of dye and 1/10 vol. of concentrated HCl—scarcely any change.

Aqueous solution of dye and 1/10 vol. of concentrated NH_4OH—orange-red.

A further test for sunset yellow FCF is the formation of orange I, described under tartrazine. A neutral or alkaline solution of sunset yellow FCF is decolorized by sodium hyposulfite (differing from tartrazine), while an acid solution is decolorized with considerable difficulty.

Sunset yellow FCF in 4 *N* hydrochloric acid is almost completely extracted by amyl alcohol. To obtain the lower sulfonated dyes, first remove sunset yellow FCF by washing the amyl alcohol extracts with equal volumes of 5% salt solution until all the dye is extracted; then dilute the amyl alcohol with an equal volume of petroleum benzine; remove the color with water, and estimate with standard titanium trichloride.

Spot Tests on Dyed Wool.—

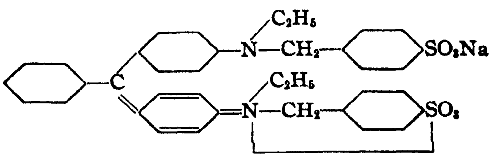
Concentrated hydrochloric acid—trifle redder.

Concentrated sulfuric acid—trifle redder.

10% Sodium hydroxide—browner.

Concentrated ammonium hydroxide—no change.

14. FD&C Green No. 1



Guinea green B ($C_{37}H_{35}N_2O_6S_2Na$) is the monosodium salt of dibenzyldiethyldiaminotriphenylcarbinol disulfonic acid anhydride. It belongs to the triphenylmethane group of dyes and is the product formed by the condensation of benzylethylaniline sulfonic acid with benzaldehyde, followed by oxidation and conversion to the monosulfonate. It is a dull, dark green powder, easily soluble in water, giving a green solution; it is also considerably soluble in 95% alcohol.

Reactions of Guinea Green B

An aqueous solution of the dye and $^1/_{10}$ volume of concentrated hydrochloric acid—yellow.

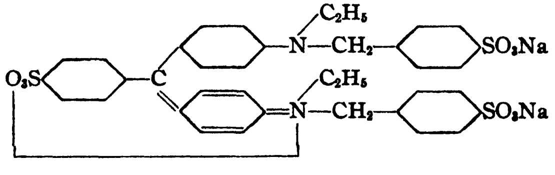
An aqueous solution of the dye and $^1/_{10}$ volume of concentrated ammonium hydroxide—decolorized.

A neutral or acid solution of the dye is decolorized by sodium hyposulfite, but the color is restored on oxidation with air. Guinea green B is readily extracted by α-dichlorohydrin, from which it may be removed with dilute alcohol after the addition of 3 volumes of carbon tetrachloride. Guinea green B is almost completely extracted by amyl alcohol from a 5% salt solution, from which it may be removed with water after addition of an equal volume of petroleum benzine. The subsidiary dye that may possibly be present in Guinea green B is malachite green (666). Its presence can easily be detected by extracting an alkaline solution of the dye with ether, washing with water until the washings are colorless, and acidifying the ether with acetic acid, which, in the presence of malachite green, will produce a green solution.

Spot Tests on Dyed Wool.—

Concentrated hydrochloric acid—yellow.
Concentrated sulfuric acid—orange.
10% Sodium hydroxide—greenish yellow.
Concentrated ammonium hydroxide—no change.

15. FD&C Green No. 2



Light green SF yellowish ($C_{37}H_{34}N_2O_9S_3Na_2$) is the disodium salt of dibenzyldiethyldiaminotriphenylcarbinol trisulfonic acid anhydride. It belongs to the triphenylmethane group of dyes and is the product formed by condensing benzylethylaniline with benzaldehyde, and then sulfonating the product to the trisulfonate, followed by oxidation and conversion into the disodium salt. The product is a reddish-brown powder, easily soluble in water, giving a green solution; it is also considerably soluble in 95% alcohol.

Reactions of Light Green SF Yellowish

An aqueous solution of the dye and $^1/_{10}$ volume of concentrated hydrochloric acid—greenish yellow.

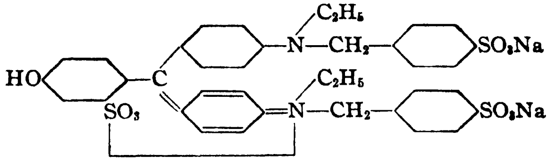
An aqueous solution of the dye and $^1/_{10}$ volume of concentrated ammonium hydroxide—almost completely decolorized.

A neutral or slightly acid solution of the dye is decolorized, by sodium hyposulfite, but the color is restored on oxidation with air. Light green SF yellowish is not readily extracted by amyl alcohol, even from 4 *N* hydrochloric acid; however, it is almost completely extracted from an aqueous solution by α-dichlorohydrin, from which it may be removed with water after the addition of an equal volume of carbon tetrachloride. Guinea green B is the lower sulfonated dye found in light green SF yellowish, and it may be estimated as follows: Extract the dye sample, containing 25% salt in solution, with several portions of amyl alcohol. Wash the solvent layer with equal volumes of a solution (250 grams of sodium chloride, 24 ml. of glacial acetic acid, 27.2 grams of sodium acetate, and enough water to make 1 liter) until no more color is extracted; dilute the amyl alcohol with petroleum benzine, remove the dye with water, and estimate it with standard titanium trichloride.

Spot Tests on Dyed Wool.—

Concentrated hydrochloric acid—yellow.
Concentrated sulfuric acid—orange.
10% Sodium hydroxide—almost colorless.
Concentrated ammonium hydroxide—almost colorless.

16. FD&C Green No. 3



Fast green FCF ($C_{37}H_{34}N_2O_{10}S_3Na_2$) belongs to the triphenylmethane group of dyes. It is similar to Guinea green B, differing only in that *p*-hydroxy-*o*-sulfobenzaldehyde is substituted for benzaldehyde. The dye is the product formed by condensing 1 mole of the aldehyde with 2 moles of benzylethylaniline monosulfonic acid, followed by oxidation and conversion into the disodium salt. Fast green FCF is a reddish or brownish-violet powder, easily soluble in water, giving a bluish-green solution; it is also considerably soluble in 95% alcohol.

Reactions of Fast Green FCF

An aqueous solution of the dye and $^1/_{10}$ volume of concentrated hydrochloric acid—greenish yellow.

An aqueous solution of the dye and $^1/_{10}$ volume of concentrated ammonium hydroxide—violet-blue.

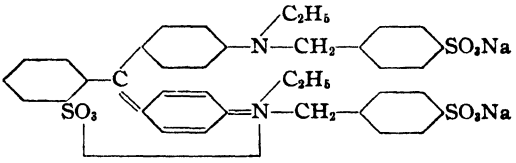
Sodium hyposulfite has no visible effect upon an alkaline solution of the dye. The neutral and acid solutions are partially decolorized, but the color is restored upon oxidation with air. The solubility of the dye in amyl alcohol and α-dichlorohydrin is similar to that of light green SF yellowish. For the determination of the lower sulfonated coloring matters present in the dye, use the method described under light green SF yellowish.

A very sensitive test for fast green FCF is the following: To about 10 ml. of an approximately 0.01% aqueous solution of the dye add 1 ml. of concentrated hydrochloric acid, 2 ml. of saturated bromine water, and an excess of a saturated solution of hydrazine sulfate. The solution will become greenish blue, which upon the subsequent addition of an excess of ammonium hydroxide changes to purplish blue, and further changes to blue upon addition of an excess of acetic acid. Light green SF yellowish becomes colorless under the same conditions.

Spot Tests on Dyed Wool.—

Concentrated hydrochloric acid—orange-yellow.
Concentrated sulfuric acid—greenish yellow.
10% Sodium hydroxide—blue.
Concentrated ammonium hydroxide—blue.

17. FD&C Blue No. 1



Brilliant blue FCF ($C_{37}H_{34}N_2O_9S_3Na_2$) also belongs to the triphenylmethane group of dyes. It is the disodium salt of dibenzyldiethyldiaminotriphenylcarbinol trisulfonic acid anhydride. The dye is the product formed by condensing one mole of benzaldehyde *o*-sulfonic acid with two moles of benzylethylaniline sulfonic acid, followed by oxidation and conversion into the disodium salt. Brilliant blue FCF is a bronze-purple powder, dissolving in water, giving a greenish-blue solution; it is also considerably soluble in 95% alcohol.

Reactions of Brilliant Blue FCF

An aqueous solution of the dye and $^1/_{10}$ volume of concentrated hydrochloric acid—greenish yellow.

An aqueous solution of the dye and $^1/_{10}$ volume of concentrated ammonium hydroxide—no change.

An aqueous solution of the dye and $^1/_{10}$ volume of 10% sodium hydroxide—not changed in the cold, but on boiling a reddish-violet color is formed.

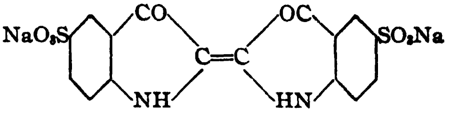
Sodium hyposulfite has no perceptible action on a neutral solution, but the acid and alkaline solutions are made momentarily lighter, the color returning almost immediately. For the solubility of the dye in amyl alcohol and α-dichlorohydrin, and the determination of lower sulfonated coloring matters present in the dye, see light green SF yellowish.

If the test with bromine described under fast green FCF is performed upon brilliant blue FCF, the color will become greenish yellow, which upon addition of excess of ammonium hydroxide changes to a purple, and upon addition of excess of acetic acid changes to a bluish red (differing from fast green FCF).

Spot Tests on Dyed Wool.—

Concentrated hydrochloric acid—yellow.
Concentrated sulfuric acid—yellow.
10% Sodium hydroxide—no change.
Concentrated ammonium hydroxide—no change.

18. FD&C Blue No. 2



Indigo carmine ($C_{16}H_8N_2O_8S_2Na_2$) is the sodium salt of the disulfonic acid of indigotin. The latter belongs to the indigoid group of coloring matters and is the product formed by the following series of chemical changes: naphthalene to phthalic anhydride—to anthranilic acid—to phenylglycine-*o*-carboxylic acid—to indoxyl carboxylic acid—and finally to indigo. Owing to its more homogeneous composition, synthetic indigo has almost entirely replaced the natural product for the manufacture of indigo carmine. Indigo carmine is a bluish-brown powder, easily soluble in water, giving a blue solution; it is only sparingly soluble in 95% alcohol.

Reactions of Indigo Carmine

An aqueous solution of the dye and $^1/_{10}$ volume of concentrated hydrochloric acid—not noticeably changed.

An aqueous solution of the dye and $^1/_{10}$ volume of concentrated ammonium hydroxide—greener.

A neutral, alkaline, or acid solution of the dye is decolorized by sodium hyposulfite, but the color is restored upon oxidation with air. Boiling a solution of the dye with dilute fixed alkalies destroys the dye, with the formation of brown isatin compounds. Indigo carmine is extracted to a large extent from a 4 *N* hydrochloric acid solution by amyl alcohol, from which it may be removed by *N*/16 hydrochloric acid. α-Dichlorohydrin extracts the dye from its aqueous solutions to a small extent. The subsidiary dye product of indigo carmine is the monosulfonated compound. This can be estimated by first removing the disulfonate from the amyl alcohol extract with *N*/16 hydrochloric acid, washing the monosulfonated dye out with water, and estimating it with standard titanium trichloride.

Spot Tests on Dyed Wool.—

Concentrated hydrochloric acid—darker.
Concentrated sulfuric acid—darker.
10% Sodium hydroxide—greenish yellow.
Concentrated ammonium hydroxide—greenish blue.

The coal-tar dyes described above, which are permitted for coloring food products, must necessarily be of exceptional purity and free from extraneous organic substances in so far as possible with good manufacturing practice. They must also be free from such harmful ingredients as lead and arsenic, whereas the tolerance permitted for other heavy metals such as iron, copper, zinc, etc., is limited to traces.

With these dyes or their mixtures, it is possible for a manufacturer to compound any and all desired color shades. The food industry should, therefore, for their own protection, be urged to use only such colors as have been permitted and certified.

III. NATURAL ORGANIC COLORING MATTERS

Nature with its polychromatic colored trees, blossoms, fruits, and foliage is the source of many coloring materials. Even the animal world contributes its share to the spectrum of organic colors. All coloring matters to be useful should possess the property of attaching themselves intimately to textiles, either with or without the use of a mordant. This combination of the coloring matter with the fibers must of necessity be of a chemical nature, and it must not be destroyed by mechanical means.

The use of natural coloring matters was doubtless known to the early Egyptians, as fabrics found in the tombs of the Pharaohs give evidence of their knowledge in dyeing with madder and indigo. At the present time, the products of the coal-tar industry have superseded to a large extent the use of natural coloring matters. As stated above, natural coloring matter may be of either animal or plant origin. To the first group belong the coloring matters of cochineal, kermes, and lac dye; in the second group may be included many different plant species. Thus, in the following table, trees, barks, fruits, leaves, blossoms, roots, and mosses are examples of the source of the coloring material with its botanical name, and the coloring principle:

TREES: brazilwood, *Caesalpinia braziliensis*, brazilin; logwood, *Haematoxylon campechianum* haematoxylin; sanderswood, *Pterocarpus santalinus*, santalin.

BARKS: quercitron bark, *Quercus tinctoria*, quercetin; quebracho bark, *Quebracho colorado*, quebracho; cinchona bark, *Cinchona succirubra*, quinotannin.

FRUITS: currants, *Ribes rubrum*, cyanidin; black grape, *Vitis vinifera*, oenin; bilberry, *Vaccinium myrtillus*, myrtillin.

LEAVES: woad, *Isatis tinctoria*, similar to that of indican; sumach, *Rhus coriaria*, gallotannin; indigo, *Indigoferae*, indigotin.

BLOSSOMS: safflower, *Carthamus tinctoria*, carthamin; pansy, *Viola tricolor*, violanin; petunia, *Petunia hybrida*, petunidin.

ROOTS: madder, *Rubia tinctoria*, alizarin; turmeric, *Curcuma longa*, curcumin; gentian, *Gentiana lutea*, gentisin.
MOSSES: orchil, *Rocella tinctoria*, orcein; litmus, *Lecanora tartarea*, azolitmin and erythrolitmin.

Consideration may also be given to the grouping of natural coloring matters according to the chemical constitution of their coloring principles. Natural coloring materials that contain derivatives of benzene are unknown. The number of known derivatives belonging to the naphthalene series is very limited, but they include the coloring matters derived from lapachol, greenheart trees, and *Lomatia longifolia*. Anthracene derivatives, on the other hand, are very numerous. They include, among others, madder, rhubarb, aloes, senna, and many other pharmacologically active substances, which, however, are not commercially considered for their coloring principle. To the anthracene type belong also the coloring matters of cochineal, kermes, and lac dye. Xanthone derivatives include the coloring matters of Indian yellow and gentian root. Some of the flavone derivatives come from poplar buds, galanga root, quercitron bark, and old fustic. The coloring matters of cornflower, rose, peony, etc., are anthocyan derivatives. Pyrrhol derivatives include the coloring matters of plant green and of logwood.

Coloring matters of natural origin that have found considerable use in coloring food products, and therefore require more consideration, are the following: alkanet, annatto, brazilwood, caramel, carotene, chlorophyll, cochineal, cocoa red, fustic, indigo, litmus, logwood, madder, orchil, Persian berries, quercitron bark, safflower, saffron, sanderswood, and turmeric.

1. Alkanet

Alkanet is obtained from the root of *Anchusa tinctoria*. Its coloring principle is alkannin ($C_{16}H_{16}O_5$). It is insoluble in water, but soluble in alcohol, ether, petroleum ether, and chloroform. Alkalies dissolve the coloring principle with the formation of a blue color. Lead acetate added to an alcoholic alkanet solution forms a blue precipitate; stannous chloride, a crimson; ferric chloride, a violet.

2. Annatto

Annatto is the fruit of a shrub, *Bixa orellana*. It contains two coloring matters: orellin, a yellow color of technically minor importance, and the principal coloring matter, bixin ($C_{25}H_{30}O_4$). Orellin is soluble in water and alcohol, but insoluble in ether; bixin is insoluble in water, only slightly soluble in alcohol, and readily soluble in ether, giving a brownish-yellow solution. Mineral acids with bixin produce a yellower shade. Alkalies dissolve bixin, giving a brownish-red solution. Annatto forms an orange lake with alumina; and with copper, a brownish yellow. A filter paper impregnated with an alkaline solution of annatto will show a pink color upon drying and treating with a drop of stannous chloride solution. A further test is the formation of a blue coloration with concentrated sulfuric acid.

3. Brazilwood

Brazilwood, *Caesalpinia braziliensis*, contains the coloring principle brazilin ($C_{16}H_{14}O_5$). This is readily soluble in water and alcohol, giving a reddish-orange solution. Hydrochloric acid added to an aqueous solution produces an orange coloration; ammonia and alkalies form a carmine-red solution. A solution of alum added to an aqueous solution of brazilwood produces a red precipitate; a solution of stannous chloride, a rose-red precipitate; and a solution of ferric chloride, a brownish-yellow precipitate. It dyes wool mordanted with chromium, reddish brown; with alumina, bluish red.

4. Caramel

Caramel is the product obtained by heating sugar above the melting point, but short of charring, when it is transformed into a stiff brown mass. Caramel is considered to be a carbohydrate of high molecular weight and a condensation or polymerization product of mono- or disaccharides. Caramel is soluble in water, giving a reddish-brown solution, but almost insoluble in 80% alcohol, ether, petroleum benzine, or chloroform. Many tests for caramel have been developed, most of them being based upon its insolubility in ether or amyl alcohol solution. Probably the most sensitive test for caramel is the Woodman and Newhall modification of Amthor's test. The procedure is as follows:

Precipitate the caramel by means of zinc chloride and potassium hydroxide and wash free from adhering material with hot water. Dissolve the precipitate with 10% acetic acid, concentrate by evaporation, neutralize, filter, and divide into two portions. To one portion add 3–5 volumes of paraldehyde and just sufficient absolute alcohol to dissolve the paraldehyde, and allow to stand. The presence of caramel is indicated by the formation of a brownish precipitate. To the other portion of the solution add an equal volume of a freshly prepared phenylhydrazine reagent (2 parts of phenylhydrazine hydrochloride, 3 parts of sodium acetate, and 20 parts of water), which produces a dark brown precipitate.

5. Carotene

Carotene is very widely distributed in nature. It derives its name from the carrot (*Daucus carota*), which contains the coloring principle carotene, ($C_{40}H_{56}$), and is an unsaturated hydrocarbon. An isomer of carotene, lycopene, has been found in the tomato, as has lutein ($C_{40}H_{56}O_2$), the yellow coloring matter of egg yolk. Willstätter and Mieg isolated carotene from green leaves; it has also been found in the leaves of wheat, grape, olive, spinach, as well as in the peel of the banana and orange. The carotene pigment occurs in nature in four isomeric forms, α, β, γ, and K, which differ only in the position of the double bond. The α-isomer is optically active while the β- and γ-modifications are optically inactive (see page 231). δ-Carotene[4] appears to be a monocyclic pigment derived from α-carotene and has so far been characterized only spectroscopically. Carotene is easily soluble in chloroform and carbon disulfide, but not easily soluble in ether and petroleum benzine. Ethyl and methyl alcohol dissolve only traces on heating. A solution of carotene in alcohol which has been diluted to 80–90% of alcohol by water will give up the color completely to carbon disulfide or petroleum benzine. A solution of carotene in oil or melted fat will give a green color on dissolving a very small crystal of ferric chloride in the melted warm fat. This test is also given by xanthophyll. Carotene produces a blue color with concentrated sulfuric acid.

---

[4] A. Winterstein, *Z. physiol. Chem.*, **219**, 249 (1933).

6. Chlorophyll

Chlorophyll is the green coloring matter of the leaves of plants, usually obtained from the stinging nettle, *Urtica dioica*. Willstätter and Stoll have found commercial chlorophyll to contain four pigments: chlorophyll *a* ($C_{55}H_{72}O_5N_4Mg$) (about 62% of the total color), a blue-black coloring matter giving a greenish-blue solution; chlorophyll *b* ($C_{55}H_{70}O_6N_4Mg$) (about 23% of the total color), a greenish-black coloring matter, giving a pure green solution; and two yellow pigments: xanthophyll ($C_{40}H_{56}O_2$) (about 10% of the total color); and carotene ($C_{40}H_{56}$) (about 5% of the total color).

A characteristic reaction of chlorophyll is the formation of a brown phase. This is produced by treating an ethereal solution of chlorophyll with half its volume of a solution containing 10% of potassium hydroxide in methyl alcohol. Immediately a change of color to brown is noted, which will gradually disappear with the formation of the original green. This procedure may be used in separating the accompanying yellow pigments, which then may be re-extracted by the ether after addition of water, while the chlorophyll remains in the alkaline solution. If, however, water is added before the brown phase disappears, part of the chlorophyll will be re-extracted by the ether. Chlorophyll is soluble in ether or absolute alcohol; it is sparingly soluble in 95% ethyl or methyl alcohol; and it is almost insoluble in petroleum benzine. A small addition of water to the methyl or ethyl alcohol solution of chlorophyll will aid solubility in the petroleum benzine.

7. Cochineal

Cochineal is a coloring matter of animal origin, and is obtained from the *Coccus cacti* insects. It contains the coloring principle carminic acid ($C_{22}H_{20}O_{13}$). It is soluble in water and alcohol, but insoluble in ether. An aqueous solution is of a scarlet-red shade, which upon the addition of alkalies or neutral lead acetate is changed to bluish red. The most characteristic test for cochineal is the formation of a pea-green coloration, produced by the addition of a saturated solution of uranium acetate to a neutral solution of carmine. Cochineal is not reduced by sodium hyposulfite. It is found in commerce generally as a lake in combination with metals such as alumina, calcium, and magnesium, and sometimes tin.

8. Cocoa Red

Cocoa red is obtained from cocoa beans. According to Schweitzer,[5] cocoa red is a decomposition product of a glucoside, cacaonin, produced during fermentation by a diastatic ferment which also produces theobromine, caffeine, and glucose. According to Reutter[6] the formula for cocoa red is $C_{40}H_{60}O_{27}N$. Cocoa red is insoluble in ether and petroleum benzine, slightly soluble in carbon disulfide, easily soluble in alcohol. The neutral solution has a violet color. Weak alkalies produce a green or bluish-green coloration, and mineral acids, a red solution. Cocoa red produces colored precipitates with salts of iron, copper, and silver.

9. Fustic

Fustic is obtained from the tree *Chlorophora tinctoria* or *Maclura tinctoria*. There are two distinct coloring matters present in fustic—morin and maclurin. Morin ($C_{15}H_{10}O_7$) is the principal color. It is sparingly soluble in water, but more readily in alcohol, giving a yellow color. Hydrochloric acid added to the aqueous solution produces no change. Sodium hydroxide gives a deep orange coloration. Ferric chloride added to an aqueous or alcoholic solution produces an olive-green color. It dyes wool mordanted with chromium olive-yellow; with alumina yellow; and with iron olive-brown. Morin is a sensitive reagent for aluminum; it gives a green fluorescence in alcoholic solution. Maclurin ($C_{13}H_{10}O_6$) is more soluble than morin in water, alcohol, and in ether. The ethereal solution gives a green and brown fluorescence. The aqueous solution is of a pale yellow shade and is not intensified greatly by the addition of alkalies. Ferric chloride produces a greenish-black coloration with an aqueous solution of maclurin. It dyes wool mordanted with alumina, chromium, or iron in shades similar to those given by morin. An aqueous solution of fustic is yellow. Hydrochloric acid produces a lighter shade; ammonia, an orange-yellow solution; sodium hydroxide, a brownish-yellow color. A solution of alum added to a neutral aqueous solution of fustic produces an orange-yellow precipitate, which dissolves in hydrochloric acid and yields a bluish-green color. A solution of stannous chloride produces a yellow precipitate; ferric chloride, a brown to olive-green precipitate; and copper sulfate, a brownish-yellow precipitate.

10. Indigo

Indigo is obtained from the plant genus *Indigofera*. The coloring principle of indigo is indigotin ($C_{16}H_{10}N_2O_2$) which is insoluble in water. By sulfonation of indigotin and formation of its sodium salt, indigo carmine is produced. The natural indigo has been largely displaced by the synthetic product. For its description and reactions see page 317.

---

[5] C. Schweitzer, *Pharm. Ztg.*, **43**, 380 (1898).

[6] L. Reutter, *Compt. rend.*, **156**, 1842 (1913).

11. Litmus

Litmus is obtained from various species of lichens, mainly *Lecanora tartarea* and *Roccella tinctoria*. The coloring principle of litmus is azolitmin ($C_7H_7NO_4$). Ether extracts azolitmin from an acid solution. The coloring matter of litmus is red, which is slightly soluble in water; the addition of alkalies, however, dissolves it, giving a blue solution. Azolitmin is insoluble in alcohol. It is chiefly used as an indicator.

12. Logwood

Logwood or campeachy wood is obtained from the tree *Haematoxylon campechianum*. The coloring principle of logwood is haematoxylin ($C_{16}H_{14}O_6$). It is sparingly soluble in cold water, but readily in hot water, alcohol, and ether. The aqueous solution is colored purple with alkalies, but on exposure to air it gradually assumes a brown tint. In combination with heavy metals haematoxylin forms strongly colored insoluble salts: the iron lake is black; the chromium lake, blue-black; the copper lake, greenish black; the alumina lake, purplish blue; and the lead lake, dark blue-violet. An aqueous solution of logwood is yellowish brown; changed by the addition of ammonia from red to violet, and by sodium hydroxide to blue, which on standing becomes brown. Hydrochloric acid added to an aqueous solution produces a reddish-yellow coloration.

13. Madder

Madder is obtained from the ground root of *Rubia tinctoria*. The coloring principles of madder are chiefly alizarin and a small quantity of purpurin. Alizarin ($C_{14}H_8O_4$) is insoluble in cold water, slightly soluble in hot water, but quite soluble in alcohol and ether, giving a yellowish-red solution. Alkalies produce a violet color. Alizarin with alumina or zinc has the property of forming salts of a brilliant red shade, and with iron those of a violet-black shade. Purpurin ($C_{14}H_8O_5$) is very sparingly soluble in water or alcohol, giving an intense yellow to reddish-yellow color. Alkalies produce a purple-red solution. Alizarin dyes cotton mordanted with alumina and lime red; with iron, violet; or with chromium, brown.

14. Orchil

Orchil is obtained from various species of lichens, mainly *Roccella tinctoria*. The coloring principle of orchil is orcein ($C_{28}H_{24}N_2O_7$) which is slightly soluble in water, giving a wine-red solution; it dissolves readily in alcohol, but is insoluble in ether. Mineral acids produce a yellower shade, while alkalies produce a blue shade. Orchil is reduced by sodium hyposulfite, but the color is restored by air oxidation. Orchil dyes, strips, and redyes wool very readily.

15. Persian Berries

Persian berries are the seed-bearing fruit of *Rhamnus amygdalinus*. It contains the glucosides of two coloring matters, rhamnetin and a small proportion of rhamnazin. Rhamnetin ($C_{16}H_{12}O_7$) is insoluble in water and only sparingly soluble in alcohol. Alkaline solutions produce a pale yellow color; alcoholic lead acetate produces an orange-red precipitate. It dyes wool mordanted with chromium, reddish brown; with alumina, brownish orange; and with iron, deep olive. Rhamnazin ($C_{17}H_{14}O_7$) is very sparingly soluble in alcohol. It dissolves in alkaline liquids to form orange-yellow solutions. Alcoholic ferric chloride added to a neutral solution produces an olive-green coloration. Rhamnazin dyes wool mordanted with chromium, golden yellow; with alumina, orange-yellow; and with iron, olive-brown. A neutral solution of Persian berries is of a yellow shade. Addition of hydrochloric acid produces no visible change, but alkali changes it to a reddish shade. In a neutral extract, a solution of alum or stannous chloride produces a golden-yellow precipitate; a solution of ferric chloride gives a dark olive-green coloration.

16. Quercitron

Quercitron is obtained from the inner bark of *Quercus tinctoria*, a species of oak tree. The coloring principle of quercitron bark is quercetin ($C_{15}H_{10}O_7$). It is the most important as well as the most abundant of the dyes of the flavone type class. It is insoluble in water but soluble in alkalies, giving a yellow color, changed to a brown by air. Quercetin forms a bright orange-red precipitate with an aqueous lead acetate solution. Alcoholic ferric chloride produces a dark green color with quercetin. It dyes wool, mordanted with alumina, brownish orange; with chromium, reddish brown; or with iron, olive-black. A solution of quercitron bark is brownish yellow. Hydrochloric acid produces a precipitate that is insoluble in ether. A solution of alum added to a neutral solution of quercitron bark produces a yellow flocculent precipitate; a solution of stannous chloride also produces a yellow precipitate; a solution of ferric salts, an olive to black precipitate; a solution of copper salts, a greenish-yellow precipitate.

17. Safflower

Safflower is obtained from the flowers of *Carthamus tinctoria*. It contains two coloring matters: one a water-soluble yellow, commercially of little value, and the other the important coloring matter carthamin ($C_{15}H_{12}O_6$). It is an orange dye insoluble in water, but readily soluble in alkaline solution, giving a red shade; mineral acids precipitate the color from basic solutions. The coloring matter of safflower is insoluble in ether, but slightly soluble in ethyl or methyl alcohol. Concentrated sulfuric acid produces a dull red coloration. Safflower dyes silk and cotton from an alkaline bath, rose-red shades.

18. Saffron

Saffron consists of the dried petals of *Crocus sativus*. The coloring principle of saffron is a glucoside, crocetin ($C_{20}H_{24}O_4$). It is very readily soluble in water or diluted alcohol, but sparingly soluble in ether or petroleum benzine. Concentrated sulfuric acid produces a blue coloration which gradually changes to violet, cherry-red, and finally to brown. Saffron dyes fabric, mordanted with alumina, orange; and mordanted with tin, yellow.

19. Sanderswood

Sanderswood, *Pterocarpus santalinus*, contains the coloring principle santalin ($C_{34}H_{28}O_{10}$).[7] Santalin is sparingly soluble in alcohol, but readily in ether with a bright red coloration having a green fluorescence. Ammonia produces a reddish-violet solution; sodium hydroxide, a dull red. An alcoholic solution of sanderswood is orange-brown, which is changed to an intense reddish violet by the addition of a few drops of ferric chloride; and by a few drops of stannous chloride, to a reddish coloration. A neutral solution is reduced by sodium hyposulfite, but the color returns upon oxidation with air. Sanderswood dyes wool and cotton, mordanted with chromium, a brownish red; with alumina, orange-red; or with iron, maroon. Wool is also dyed direct, a brick-red shade.

20. Turmeric

Turmeric is the underground stem of *Curcuma longa* and contains the coloring principle curcumin, $C_{21}H_{20}O_6$. Curcumin is sparingly soluble in hot water, but more readily so in hot alcohol. It is also soluble in ether, producing a strong green fluorescence. Alkali added to a solution of curcumin forms a reddish-brown solution unstable in air. Hydrochloric acid added to a solution of curcumin gives an orange or reddish-orange solution. The behavior of curcumin with boric acid is the most characteristic reaction. If an alcoholic solution of turmeric or curcumin is mixed with boric acid, a deep red color is formed, distinct from that produced by alkali. Schlumberger's "resocyanine" is the product formed by heating a dilute alcoholic solution of curcumin with boric acid and sulfuric acid, which gives a deep red color. This changes slowly in the cold, but rapidly on heating, to dark red, orange, and finally to yellow. A mixture of boric acid and hydrochloric acid imparts to turmeric paper a reddish-brown color which is turned bluish green by ammonia. From an acid bath, turmeric dyes cotton, wool, and silk a greenish yellow.

Standardization of the natural coloring material has not been undertaken thus far, owing to the difficulty encountered in evaluating quantitatively the coloring principles of most of these substances. Efforts have therefore been confined chiefly to restricting and limiting the amount of harmful inorganic constituents, such as arsenic, lead, copper, zinc, etc. All natural coloring substances are in almost every case mixtures of heterogeneous compositions, which are difficult to separate. They generally contain more than one coloring principle, one of these being technically of importance, while the other or others are considered as ballast material (annatto, safflower, madder). Then again, the percentage of the active color principle is variable, depending upon the soil, the time of harvesting, and after-treatments. These and other imponderables make it well-nigh impossible to obtain uniformity of color shades with definite quantities of vegetable colors. It is mainly in localities where coal-tar dyes for food-coloring purposes are prohibited, that vegetable colors are used.

---

[7] I. H. Raudnitz, M. Navratil, and P. Benda, *Ber.*, B67, 1036 (1934).

Selected Bibliography

Federal Security Agency, Food and Drug Administration, Service and Regulatory Announcements, Food, Drug and Cosmetics No. 3, *Coal-Tar Color Regulations.* Washington, D. C., 1940.

Jacobs, M. B., *Chemical Analysis of Foods and Food Products.* Van Nostrand, New York, 1938.

Mayer, F., *Natural Coloring Matters.* Reinhold, New York, 1943.

Methods of Analysis. Assoc. Official Agr. Chemists, Washington, D. C., 1940.

Rowe, F. M., *Colour Index.* Society of Dyers and Colourists, Bradford, Yorkshire, 1924.

Woodman, A. G., *Food Analysis.* McGraw-Hill, New York, 1942.

Chapter X

THE DIGESTION AND FATE OF FOODSTUFFS

Alfred E. Wilhelmi
New Haven, Connecticut

I. NATURE AND SITE OF THE DIGESTIVE PROCESSES

Most of the chemical reactions in the body take place in aqueous solution, and the basis of the systems of transport is the diffusion of molecules in solution across tissue spaces and through cell membranes. Before they can be absorbed and utilized by the tissues, the useful components of foodstuffs must be simplified and brought into solution by the processes of digestion. These processes involve: (*1*) the *mechanical breakdown* of the food masses, which allows thorough mixing with the digestive juices and increases the surface for enzyme action; (*2*) the *chemical breakdown* by hydrolytic enzymes of large, relatively insoluble molecules (polysaccharides, proteins, and lipids) into smaller, soluble, and diffusible molecules; (*3*) the *solution* of these products of digestion, and of the inorganic salts and organic extractives of the foods, in the digestive fluids; (*4*) the *absorption* of substances in solution by the intestinal epithelium; and (*5*) the *excretion* of waste materials which accumulate in the large intestine.

Digestion takes place in the alimentary canal (Fig. 21), which may be regarded simply as a muscular tube lined with a glandular epithelium. Its most important parts are the mouth, pharynx, and esophagus, the stomach, the small intestine (duodenum, jejunum, and ileum), and the large intestine, rectum, and anus. Each of these parts differs from the others in structure and function, and together with their vascular, lymphatic, and nervous supplies they form a coordinated functional unit. Although emphasis in this account is placed on the chemistry of digestion, it is important to remember that none of the digestive processes is independent of the other processes and functions of the alimentary canal.

The important accessory digestive glands are: (*1*) the salivary glands (parotid, submaxillary, and sublingual) opening into the mouth; (*2*) the pancreas, with its ducts opening into the duodenum; and (*3*) the liver and gall bladder, with a common duct opening into the duodenum. Their secretions are complementary to the secretions of the digestive tract. Together they provide the digestive enzymes, insure the maintenance of the proper hydrogen-ion concentrations for enzymatic action, and supply the fluids in which the products of digestion must be dissolved before they can be absorbed and transported to the tissues of the body.

Since digestion is extracellular, taking place almost entirely within the lumen of the alimentary canal, the primary stimuli to the digestive processes must arise from the foodstuffs themselves. The necessity for making rapid responses to the physical and chemical stimulus of ingested foodstuffs requires extensive nervous and hormonal controls to coordinate the activities of the digestive tract. Some of these controls are described in the following sections, but a more vivid account of them can be found in the studies of Pavlov and his coworkers[1a] on the activities of the digestive glands. Digestion involves the considerable participation of both body and mind; it begins with the anticipation of a meal, and it is most tranquil when due regard is given to the preparation, appearance, odor, and savor of the food, and when meals are taken with gusto and in peace.

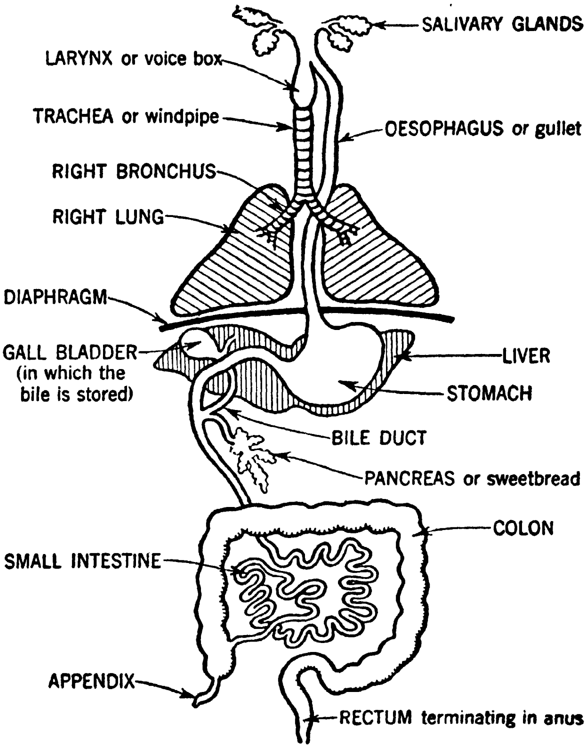


Fig. 21.—The alimentary canal and its accessory digestive glands.[1]

---

[1] Lancelot Hogben, *Science for the Citizen*. Allen & Unwin, London, 1938, p. 786.

[1a] I. P. Pavlov, *The Work of the Digestive Glands*. Griffin, London, 1910.

II. SALIVARY DIGESTION

1. The Saliva

The salivary glands produce a mixed secretion, the composition of which is indicated in Table 32. The normal secretion is neutral or slightly alkaline (because of the presence of bicarbonate), and it may contain traces of many substances present in the blood. The most important proteins in the secretion are mucin and the enzyme, salivary amylase (diastase, ptyalin).

TABLE 32

COMPOSITION OF NORMAL HUMAN SALIVA[a]

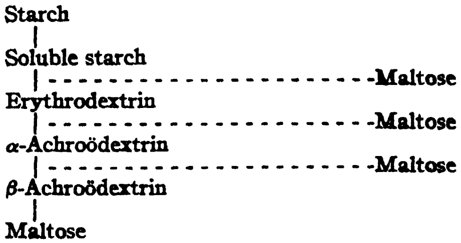
(Quantities per 100 ml. of saliva)

Component	Normal range
*p*H	6.0–7.9
Titratable alkalinity (as 0.02 *N* HCl)	90–190 ml.
Ammonia N	2– 10 mg.
Total calcium, as Ca	4– 8 mg.
Inorganic phosphate, as P	10– 25 mg.
Chloride, as Cl	30– 60 mg.
Carbonate, as CO_2	20– 45 ml.
Protein	200–400 mg.

[a] P. B. Hawk and O. Bergheim, *Practical Physiological Chemistry.* Blakiston, Philadelphia, 1937.

Mucin, which is secreted by many glands throughout the alimentary canal, is a mixture of a glycoprotein and a mucopolysaccharide.[2] See page 172. Its solutions are viscous and sticky. It acts as a lubricant, protects the epithelium of the alimentary tract, and assists in binding food particles into small masses convenient for swallowing.

Salivary amylase catalyzes the hydrolysis of starch to maltose. Its primary action on native starch is to convert it into a soluble form. The exact nature of this step, and of most of the subsequent steps in starch hydrolysis, is not understood, largely because of lack of information about the structure of starch, and because of difficulties in determining the constitution of the intermediate products of hydrolysis. The course of starch hydrolysis by amylase may be outlined as follows:



"Erythrodextrin" is so called because it reacts with iodine to produce a red color. The other dextrins do not give colors with iodine; they probably contain less than eight glucose units per molecule, and differ, as between the α- and β-forms, in their state of aggregation.[3] Maltose is a reducing disaccharide, and since it is set free at each stage of the hydrolysis, the course of the reaction may be followed by measuring the reducing power of the solution. Salivary amylase acts optimally at or near *p*H 7.0. Its action is enhanced by chloride, bromide, or nitrate ions, and it is inactive in their absence. Free hydrochloric acid above 0.005% inhibits the enzyme. In the stomach it is ultimately destroyed by the hydrochloric acid of the gastric juice.

---

[2] Karl Meyer, *Symposia on Quantitative Biology*, 6, 91 (1938).

2. Salivary Secretion

The salivary glands are controlled by the nervous system. Since the sight and odor or the thought of food, or stimuli associated with food, can elicit a secretion of saliva, the higher nervous centers may influence the secretion. Mechanical and chemical stimulation of the nerve endings in the mouth also excites the secretion of saliva. The response is adapted to the nature of the stimulus. Food calls forth a saliva rich in organic matter, while acids and alkalies, or sand, elicit a watery secretion, poor in organic matter, which washes out the mouth. This flexibility of response is characteristic of the glands of the digestive tract. It emphasizes the fact that the composition of a meal determines the pattern of digestive activity.

In addition to its digestive function, the saliva moistens the tissues of the mouth and pharynx, cleanses the region of food and foreign particles, protects the membranes of the mouth and throat, acts as a solvent, and provides fluid (1 to 1.5 liters per day) for the dilution and transport of materials to the stomach.

3. Digestion in the Mouth

The oral stage of digestion is limited to mechanical breakdown, and this varies according to individual habits of chewing. It is important, since moderate chewing of food insures adequate mixing with saliva thus facilitating the action of amylase, and assists in stimulating gastric secretion. Since food spends little time in the mouth, the action of salivary amylase occurs mainly in the stomach.

III. GASTRIC DIGESTION

1. The Functions of the Stomach

As food enters the stomach (Fig. 22) the organ slowly distends. A series of gentle contractions begins near the upper end and sweeps over the body toward the pylorus. As digestion proceeds, the contractions increase in frequency, scope, and amplitude, and are accompanied by the intermittent relaxation of the muscular valve (the pyloric sphincter) separating the stomach from the duodenum. With each opening of the sphincter, a portion of the mixture of digestive secretions and partially digested food (the *chyme*) is ejected. When gastric digestion is at its height, the rate of emptying is about 10 ml. per min., but the rate may vary with the state of the material in the stomach and with the progress of digestion in the small intestine. During vigorous contractions of the stomach, the mass of material in the body of the organ is relatively undisturbed. With successive contraction waves, material is transferred from the periphery of the central mass to the wall of the stomach. It is here that mixture with the gastric secretions occurs. The semiliquid mixture moves along the stomach wall and is finally ejected through the pylorus. When the stomach is partly full, newly swallowed food falls into the central mass, and is only slowly admixed with the gastric contents. Even the mixing of liquids in the stomach is incomplete. In human subjects given 50% glucose solution, the glucose concentration in the center of the stomach is, after some minutes, still 40 to 50%, while along the stomach wall it is about 13%, and in the small intestine it is further reduced to about 7%. The stomach is not so much a mixing organ as a place of temporary storage, digestion, and dilution.

---

[3] I. A. Preece, *J. Incorporated Brewers' Guild*, **27**, 19 (1941).

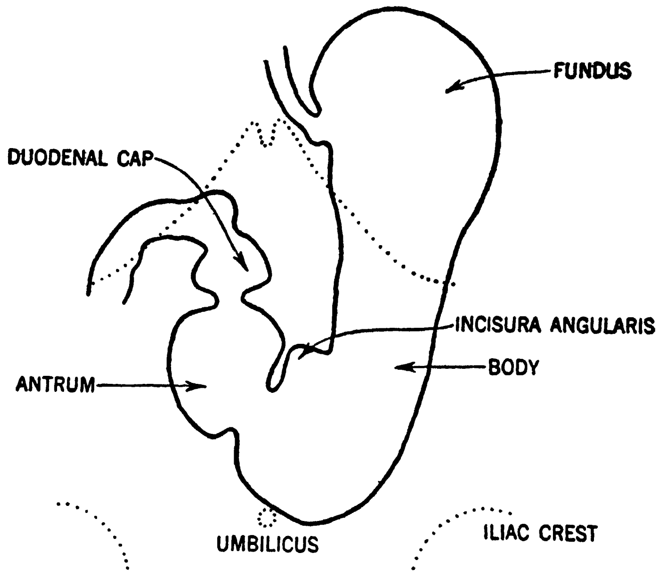


Fig. 22.—Diagram of the human stomach. The antrum and duodenal cap comprise the region of the pyloric glands.[30]

2. Gastric Secretion

Like the salivary secretion, the gastric secretion may be influenced by purely psychic stimuli. Although the presence of food in the mouth is the usual stimulus to this *cephalic* phase of gastric secretion, the volume and rate of secretion are affected by fondness for or dislike of the food being taken (Fig. 23). This phase is transitory and does little more than initiate digestion. Its onset is delayed for several minutes after the first stimulus, which offers an opportunity for the continued action of salivary amylase in the stomach.

---

[30] C. H. Best and N. B. Taylor, *The Physiological Basis of Medical Practice.* Wood, Baltimore, 1936, p. 77.

The presence of food in the stomach, particularly of the products of protein digestion or of the extractives of meat, is responsible for the maintenance of gastric secretion. It is now thought that the action of these excitatory substances (secretagogues) on the cells of the pyloric region of the stomach liberates into the blood stream a gastric hormone, *gastrin*, which excites the secretion of the glands in the fundus of the stomach.[5, 6] This humoral mechanism reinforces the nervous mechanisms during the long *gastric* phase of secretion. Another series of stimuli is evoked by the same or similar secretagogues acting in the small intestine. The mechanism of this *intestinal* phase of gastric secretion is probably a humoral one as well.

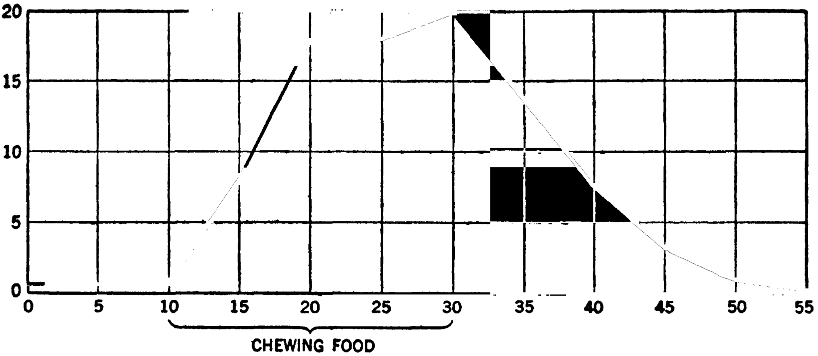


Fig. 23.—The "psychic" secretion of the gastric juice in man.

The juice was collected at 5-min. intervals during the chewing of food for 20 min., and afterward until the secretion subsided. The rise in rate of secretion during the last 5 minutes' mastication was due to the subject's particular fondness for the dessert. (After Carlson.[4])

There is at least one inhibitory phase of gastric secretion. Fats or fatty acids in the small intestine release from the intestinal wall the hormone, *enterogastrone*, which inhibits gastric motility and secretion.[5, 7] Enterogastrone has been isolated in purified form from the intestinal epithelium of hogs, and its inhibitory action has been fully verified. Thus the time of digestion may be prolonged by the action of the most difficultly digestible of the foodstuffs.

These mechanisms clarify only in part a fundamental characteristic of the gastric secretion first emphasized by Pavlov: that the secretion alters selectively in response to different foodstuffs (Table 33). The forces responsible for this selective behavior are not yet understood. Probably no single factor is an absolute determinant of gastric activity; the balance and interplay of many nervous and humoral mechanisms, each governed by the chemical nature and state of digestion of the food, must determine the elegant adaptation of the gastric secretion.

---

[4] A. J. Carlson, *The Control of Hunger in Health and Disease.* Chicago Univ. Press, Chicago, 1916.

[5] A. C. Ivy, *Glandular Therapy and Physiology.* Am. Med. Assoc., Chicago, 1942; Chap. XXX, p. 523.

[6] R. A. Gregory and A. C. Ivy, *Quart. J. Exptl. Physiol.*, **31**, 111 (1941).

[7] J. P. Quigley, *Am. J. Digestive Diseases Nutrition*, **8**, 363 (1941).

TABLE 33

RELATIVE VARIATIONS IN VOLUME, TIME OF SECRETION, TOTAL PEPTIC ACTIVITY, AND MEAN ACID STRENGTH OF CANINE GASTRIC JUICE IN RESPONSE TO MEALS OF EQUIVALENT NITROGEN CONTENT AND DIFFERENT COMPOSITION

(The smallest value in each category is taken as 100%)

Meal	Volume	Time	Total enzyme	Mean acid strength
Flesh, 100 g.	124	133	198	125
White bread, 250 g.	124	167	469	100
Milk, 600 ml.	100	100	100	135

3. The Gastric Juice

(*a*) *Composition and Origin*

The mingled secretions of the gastric glands were excellently described by William Beaumont:

> "Pure gastric juice, when taken directly out of the stomach of a healthy adult, unmixed with any other fluid, save a portion of the mucus of the stomach with which it is most commonly, and perhaps always combined, is a clear, transparent fluid; inodorous; a little saltish; and very perceptibly acid. Its taste, when applied to the tongue, is similar to thin mucilaginous water, slightly acidulated with muriatic acid. It is readily diffusible in water, wine or spirits; slightly effervesces with alkalies; and is an effective solvent of the *materia alimentaria*. It possesses the property of coagulating albumen, in an eminent degree; it is powerfully antiseptic, checking the putrefaction of meat; and effectually restorative of healthy action, when applied to old fetid sores and ulcerating surfaces."[8]

In modern times Carlson[4] has made similar observations on a subject with a gastric fistula. The composition of pure human gastric juice as he obtained it is indicated in Table 34.

Gastric juice is a mixed secretion of three main types of gland cells. The parietal cells of the gastric glands secrete practically pure hydrochloric acid.[9] The chief cells of these glands produce the enzyme pepsin. The mucous glands of the stomach produce mucin. Since the daily output of the secretion may be 2.5 liters, it is an important source of fluid in which digested material is dissolved and diluted.

---

[8] William Beaumont, *Experiments and Observations on the Gastric Juice and the Physiology of Digestion*. Peter Smith, New York, 1941. William Beaumont (1785–1853) was a U. S. Army surgeon. In 1822–1825 he had the skill, patience, and good fortune to restore to health a Canadian trapper, Alexis St. Martin, who had suffered a severe shotgun wound of the chest and abdomen. In healing, the wound left a permanent gastric fistula, and Beaumont, in the period 1825–1833, made many observations on the gastric juice of his patient. His book, recording these experiments, was published in 1833, and today, after 110 years, it is a fresh and shining record of honest observation and critical understanding.

[9] J. S. Gray and G. R. Bucher, *Am. J. Physiol.*, **133**, 542 (1941).

TABLE 34

COMPOSITION OF HUMAN GASTRIC JUICE

*p*H	0.9–1.2
Free hydrochloric acid	0.40–0.50%
Total acidity	0.45–0.60%
Total solids	0.55–0.60%
Organic	0.42–0.46%
Inorganic	0.13–0.14%
Total nitrogen	0.051–0.075%
Specific gravity	1.006–1.009

(*b*) *Special Components*

Hydrochloric Acid.—The mechanism of the production in the stomach of a strong mineral acid in high concentration (0.165 *N*) has attracted the attention of physiologists for many years, with little result but a profusion of theories. The most recent and hopeful theory rests upon the discovery by Davenport and Fisher[10] of high concentrations of carbonic anhydrase in the parietal cells of the stomach. This enzyme catalyzes the reaction:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$

Briefly stated, the hypothesis proposes that carbon dioxide, generated by the oxidative metabolism of the parietal cells, is rapidly hydrated to carbonic acid by carbonic anhydrase. Hydrogen ions arising from the ionization of carbonic acid or some equivalent source are secreted into the lumen of the gland by a special mechanism (as yet unknown), and they are accompanied passively by chloride ions derived from the blood. Bicarbonate ions, readily available in the parietal cells, diffuse into the blood to replace the chloride ions. These events are illustrated diagrammatically in Fig. 24. The "carbonic anhydrase" theory is admirably discussed in a recent review by Gray.[11] It is not yet complete, but it has already withstood some experimental tests, and it offers a practicable line of investigation not only of the problem of acid secretion but also of the problem of how the work of secretory glands is done.

The functions of hydrochloric acid are threefold. It is partly responsible for the bacteriostatic action of gastric juice; it renders protein more susceptible to enzyme action; and it activates the proteolytic enzyme pepsin and secures a hydrogen-ion concentration suitable for peptic activity.

Mucus.—The general properties of mucin have been discussed. In the stomach, mucus protects the epithelium from the strongly acid secretion. Since mucin can combine with many equivalents of acid, it may also be useful in regulating the acidity of the gastric contents.

Gastric Enzymes.—The enzymes rennin, lipase, and pepsin are secreted in the stomach. Rennin is found in the stomachs of young animals. It is a milk-clotting enzyme, converting the casein of milk into paracasein, which combines with calcium ions in the gastric juice to form insoluble calcium paracaseinate, a soft, fluffy curd which is more readily attacked by pepsin. In the young, both acid secretion and peptic activity are weak. Rennin, by coagulating milk, procures a longer digestion time for the chief milk protein, since the unchanged liquid would otherwise pass quickly through the stomach. The *p*H optimum for rennin is 6.0–6.5, in keeping with the feeble acid-secreting properties of stomachs in which it is found. It is probably secreted as inactive prorennin, and is activated by the gastric acid.

---

[10] H W Davenport and R B Fisher, *J Physiol*, **94,** 16P (1938)

[11] J. S. Gray, *Federation Proc*, **1,** 255 (1942).

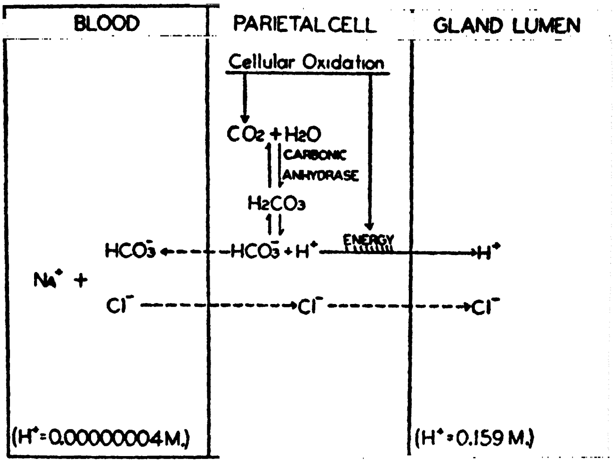


Fig. 24.—Diagram of the secretion of hydrochloric acid by the parietal cells, according to the "carbonic anhydrase" theory.

The lipolytic enzyme of gastric juice acts best at about *p*H 5.0, and is inhibited by low concentrations of free mineral acid. Except in the young, gastric lipase probably cannot effect much hydrolysis of neutral fat in the stomach.

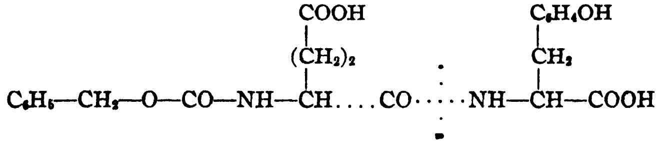
Pepsin, the proteolytic enzyme of gastric juice, is secreted as its inactive precursor, pepsinogen. Both forms of the enzyme have been isolated as crystalline proteins and have been studied in detail.[12] Pepsinogen is activated by hydrochloric acid and also by pepsin, in an autocatalytic reaction. The mechanism of activation may involve an unfolding of the protein molecule to disclose the active groups of the enzyme. The *p*H optimum of pepsin varies with its substrate; with many proteins it is near *p*H 2.2, but with the synthetic peptide carbobenzoxy-glutamyl-tyrosine it acts best at *p*H 4.0. The enzyme, like rennin can coagulate milk. Pepsin attacks both native and denatured proteins, and simpler peptides, provided they contain certain specific chemical configurations. See page 210.

---

[12] J. H. Northrop, *Crystalline Enzymes*. Columbia University Press, New York, 1938.

Due largely to the work of Max Bergmann and his associates[13, 14] on the hydrolysis of synthetic peptides of known constitution by proteolytic enzymes, it is possible to describe the action of peptidases in terms of the chemical constitution of their substrates rather than in terms of the size of the substrate molecules. The main provisions of this modern view are: (*1*) Enzymes hydrolyzing peptide bonds are classed as (*a*) *endopeptidases*, which can split peptide linkages either within or at the end of peptide chains, or (*b*) *exopeptidases*, which can only split the terminal bond of a chain; (*2*) the action of a peptidase depends on (*a*) a particular atomic arrangement in a side group of the substrate molecule, (*b*) the presence, near the specific side group, of definite chemical structures in the backbone of the substrate molecule, and (*c*) the natural optical configuration in the amino acids involved in the specific side and backbone groups. See page 212.

According to this view, the specificity of pepsin may be outlined as follows: (*1*) Pepsin is an endopeptidase; (*2*) the hydrolyzed bond must have tyrosine or phenylalanine as a member (side-group specificity); (*3*) there must be at least one free carboxyl group in close proximity to the side group (backbone specificity); (*4*) there must be another peptide bond adjacent to the hydrolyzed bond (that is, the smallest possible substrate for pepsin is a tripeptide); (*5*) the enzyme attacks the peptide bond involving the amino group of the aromatic amino acid. These points are illustrated in a typical synthetic substrate for pepsin, carbobenzoxy-*l*-glutamyl-*l*-tyrosine:



$$\mathrm{C_6H_5{-}CH_2{-}O{-}CO{-}NH{-}\overset{\displaystyle\overset{\displaystyle\overset{COOH}{|}}{(CH_2)_2}}{\overset{|}{CH}}\ldots CO\,\vdots\cdots NH{-}\overset{\displaystyle\overset{\displaystyle\overset{C_6H_4OH}{|}}{CH_2}}{\overset{|}{CH}}{-}COOH}$$

Since proteins vary widely in amino acid constitution, their digestion by pepsin will vary, in accordance with the presence or the degree of modification of the specific groups, and with the frequency of occurrence of these groups in the substrate molecule. Since the required groups recur infrequently, and only rarely at the ends of peptide chains, the products of digestion by pepsin are usually rather large peptides—the so-called "proteoses" and "peptones" in the classical terminology.

4. Digestion in the Stomach

The delay in the onset of gastric secretion, the circumstance that the ingested food is undisturbed for some time, and the influence of carbohydrate on the secretion of gastric acid (Table 33) may make possible an appreciable digestion of starch in the stomach. Beazell[15] has shown that in $^1/_2$ to 1 hr. after taking an average meal, about 40% of the starch remaining in the stomach has been hydrolyzed to maltose, and that

---

[13] Max Bergmann, *Advances in Enzymology*, Vol. 2. Interscience, New York, 1942, p. 49.

[14] Max Bergmann and J. S. Fruton, *Advances in Enzymology*, Vol. 1. Interscience, New York, 1941, p. 63.

[15] J. M. Beazell, *Am. J. Physiol.*, 132, 42 (1941).

1 hr. after taking a finely divided meal of the same composition, about 20% of the starch was present as maltose. The stomach is usually regarded as a place primarily of protein digestion, but it may be equally important in the digestion of carbohydrate.

Besides chemical constitution, the main factors determining the extent of protein digestion in the stomach are the concentration of pepsin and time (Table 33). In Beazell's experiments, the extent of peptic digestion (expressed as nitrogen no longer precipitable by 2.5% tungstic acid) was much less, in 2 hrs., than the extent of starch hydrolysis in $^1/_2$ hr. The method, however, could not detect the extensive partial digestion which almost certainly occurred. Perhaps the most important result of peptic digestion is the loosening of tissue structure which permits the escape of cell contents and the dispersion and partial solution of the food particles: the "chymification" of the gastric contents.

There is little digestion of lipid in the stomach. Fat is set free from the disintegrating tissues, and it may be partly emulsified by mechanical agitation and the action of acid and warmth.

The time of gastric digestion depends on the size and composition of the meal and on individual disposition. There are "fast" and "slow" stomachs, and, in addition, gastric activity is powerfully affected by emotional states. On the whole, an average meal requires from 2 to 4 hrs. to leave the stomach. Some hours later, the empty organ begins the intermittent, powerful contractions which are felt subjectively as "hunger pangs."

IV. INTESTINAL DIGESTION

1. The Course of Digestion in the Intestine

In the small intestine the acid chyme is partly neutralized by the secretion of alkaline fluids. In this process an array of digestive agents is produced from the pancreas, the liver and gall bladder, and the intestinal glands. The intestine begins active muscular contractions (peristalsis), in the course of which the intestinal contents are thoroughly mixed, brought into continuous intimate contact with the intestinal epithelium, and slowly moved toward the large intestine. In 5 or 6 hrs., the chyme has reached the large intestine, digestion has been completed, and practically all of the useful components of the meal have been dissolved and absorbed, together with a large proportion of the digestive secretions.

2. Pancreatic Secretion

(a) *The Control of Pancreatic Secretion*

The presence of acid or of the products of gastric digestion in the small intestine elicits a vigorous and sustained secretion of pancreatic juice. Bayliss and Starling, in 1902, first showed that this is due to a humoral mechanism. An excitatory substance, named *secretin*, is liberated from the intestinal mucosa and passes in the blood to the pancreas, eliciting a strong secretion of pancreatic juice.[16, 17] With different foodstuffs there are different and characteristic patterns of volume and rate of pancreatic secretion, and of total enzymatic activity (Table 35). The pancreas, like the stomach, responds with some precision to differences in the composition of the materials in the intestine. It does not seem likely that secretin alone can account for the character of the secretion. It is possible that the hormone secures a sustained secretion, the composition of which is determined by many other nervous and humoral factors.

TABLE 35

VARIATIONS IN THE PANCREATIC SECRETION OF DOGS IN RESPONSE TO DIFFERENT FOODSTUFFS OF EQUIVALENT NITROGEN CONTENT

(After Pavlov)

Meal	Volume, ml.	Duration, hr. min.	Total peptidase, units	Total amylase, units	Total lipase, units
Meat, 100 g.	131.6	4 12	1502	648	3600
Bread, 250 g.	162.4	7 35	1978	1601	800
Milk, 600 ml.	47.7	4 30	1085	432	4334

(*b*) *Pancreatic Juice*

The cells of the pancreas produce daily about 700 ml. of a clear, colorless, strongly alkaline fluid of the composition indicated in Table 36. The alkalinity of pancreatic juice, like that of all the intestinal secretions, is due to its high concentration of sodium bicarbonate. It can neutralize approximately an equal volume of gastric juice. Since the pancreatic enzymes operate best in neutral or alkaline solution, the neutralization of the acid chyme is one of the important functions of pancreatic juice.

TABLE 36

COMPOSITION OF HUMAN PANCREATIC JUICE

*p*H	about 8.0
Total alkalinity	0.53%
Total solids	1.3–1.4%
Organic	0.6–0.7%
Inorganic	0.7%
Specific gravity	1.008

---

[16] A. C. Ivy, *Glandular Therapy and Physiology*. Am. Med. Assoc., Chicago, 1942; Chap. XXX, p. 523.

[17] H. Greengard and A. C. Ivy, *Am. J. Physiol.*, **124**, 427 (1938). Secretin is the best known of the gastrointestinal hormones, but its nature and mode of action are still uncertain. It has been isolated as a crystalline salt. There is a question whether it is a polypeptide of molecular weight about 5000, or a simple weak base associated with the large molecule.

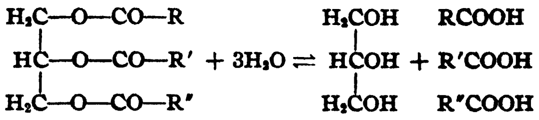
3. Pancreatic Enzymes

(a) *Amylase* (*Amylopsin*)

The amylase of pancreatic juice is somewhat more powerful than salivary amylase, but its properties and action are almost identical with it. Under its influence, the starch in the intestine is rapidly and completely hydrolyzed to maltose.

(b) *Lipase* (*Steapsin*)

Pancreatic lipase is a powerful enzyme which hydrolyzes both triglycerides and mono esters of fatty acids, in accordance with the following equations:



$$\begin{array}{l} H_2C{-}O{-}CO{-}R \\ \quad| \\ HC{-}O{-}CO{-}R' \\ \quad| \\ H_2C{-}O{-}CO{-}R'' \end{array} + 3H_2O \rightleftharpoons \begin{array}{l} H_2COH \\ \quad| \\ HCOH \\ \quad| \\ H_2COH \end{array} + \begin{array}{l} RCOOH \\ R'COOH \\ R''COOH \end{array} \qquad (1)$$

$$X{-}O{-}CO{-}R + H_2O \rightleftharpoons XOH + RCOOH, \qquad (2)$$

where R, R′, and R″ represent fatty acid chains, and X represents a monohydric alcohol. The *p*H optimum of the reaction is about 7.1. Since lipids are difficultly soluble in water, special circumstances are required for their hydrolysis. First, the surface of the substrate must be increased, to allow engagement at many points with the active groups of the enzyme. This is achieved by the emulsification of the fats by the bile salts, and perhaps other agents in the bile, which have the property of lowering surface tension. Second, at the points of attack of the enzyme, a temporary intimate association must be established between the insoluble substrate, the elements of water, and the soluble enzyme. It is probable that the bile salts, which also have the property of forming water-soluble complexes with insoluble organic substances, are concerned in this process, since the bile salts increase the action of lipase more than is to be expected from their emulsifying action alone. Both the bile and the pancreatic juice are necessary for the digestion of lipids. In the absence of either one, a large proportion of ingested fat is lost in the feces.

(c) *Peptidases*

Three peptidases of pancreatic origin have been isolated, purified, and characterized. It is now realized that there are many more peptidases in pancreatic juice, and, until the full range of peptidase activity is understood, it is clear that knowledge of protein digestion in the intestine must be incomplete.

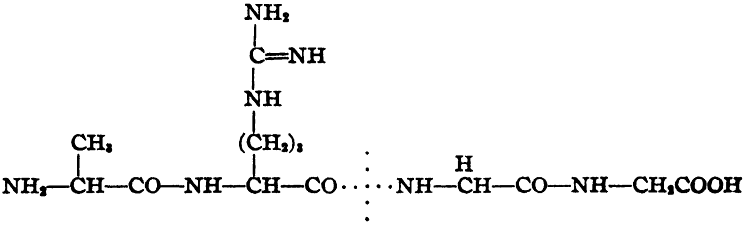
Trypsin, and its inactive precursor, trypsinogen, chymotrypsin and chymotrypsinogen, and a single carboxypeptidase, have been isolated as crystalline proteins.[12, 13] In the intestine, trypsinogen is converted to trypsin by another enzyme, enterokinase, secreted in the intestinal juice. Trypsin, in turn, converts chymotrypsinogen to

---

[13] Max Bergmann, *Advances in Enzymology*, Vol. 2. Interscience, New York, 1942, p. 49.

chymotrypsin. The inactive form of carboxypeptidase may be activated by trypsin. Each of these enzymes exhibits characteristic chemical specificities, which may be summarized as follows:

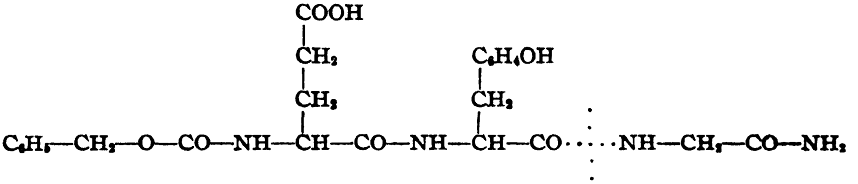
1. Trypsin is an endopeptidase, hydrolyzing native proteins and peptides at interior bonds in the peptide chain which have the characteristics of the synthetic peptide, *l*-alanyl-*l*-arginylglycylglycine:



$$\mathrm{NH_2{-}\overset{\overset{CH_3}{|}}{C}H{-}CO{-}NH{-}\overset{\overset{\overset{\overset{\overset{\overset{\overset{\overset{NH_2}{|}}{C{=}NH}}{|}}{NH}}{|}}{(CH_2)_3}}{|}}{C}H{-}CO\cdots\vdots\cdots NH{-}\overset{H}{C}H{-}CO{-}NH{-}CH_2COOH}$$

(*a*) there must be an unmodified arginine or lysine side chain; (*b*) there must be a peptide bond adjacent to the hydrolyzed bond; (*c*) the hydrolyzed bond always involves the carboxyl group of the basic amino acid.

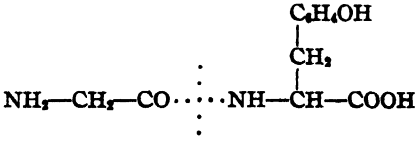
2. Chymotrypsin is also an endopeptidase, attacking peptides with the characteristics of carbobenzoxy-*l*-glutamyl-*l*-tyrosylglycine amide:



$$\mathrm{C_6H_5{-}CH_2{-}O{-}CO{-}NH{-}\overset{\overset{\overset{\overset{\overset{\overset{COOH}{|}}{CH_2}}{|}}{CH_2}}{|}}{C}H{-}CO{-}NH{-}\overset{\overset{\overset{\overset{C_6H_4OH}{|}}{CH_2}}{|}}{C}H{-}CO\cdots\vdots\cdots NH{-}CH_2{-}CO{-}NH_2}$$

(*a*) there must be an unmodified tyrosine or phenylalanine side chain; (*b*) there must be peptide bonds immediately adjacent to the hydrolyzed bond; (*c*) carboxyl groups in close proximity to the hydrolyzed bond inhibit the enzyme; (*d*) the hydrolyzed bond involves the carboxyl group of the aromatic amino acid. (Pepsin attacks this substrate, at the bond involving the amino group of the aromatic amino acid.)

3. Carboxypeptidase attacks peptides only at the end of the chain, where there must be a free amino group and the side chain of tyrosine or phenylalanine, as in glycyl-*l*-tyrosine:



$$\mathrm{NH_2{-}CH_2{-}CO\cdots\vdots\cdots NH{-}\overset{\overset{\overset{\overset{C_6H_4OH}{|}}{CH_2}}{|}}{C}H{-}COOH}$$

There are other carboxypeptidases in pancreatic juice, but none has yet been isolated and characterized.

The peptidases, like the other pancreatic enzymes, act best in alkaline solution (*p*H 7.0–8.0), but in any particular instance the *p*H optimum depends on both enzyme and substrate. A puzzling fact is that the *p*H of the intestinal contents is found to be less than 7.0 throughout most of the intestine. This means either that the pancreatic enzymes work at a disadvantage or, more likely, that the region of important digestive activity is the immediate neighborhood of the intestinal mucosa, where the alkaline secretion of the intestinal glands may secure an adequate hydrogen-ion concentration for rapid enzymatic action.

4. The Extent and Relative Importance of Pancreatic Digestion

Digestion by the pancreatic enzymes leads to the complete hydrolysis of starch to maltose, the extensive hydrolysis of lipids to fatty acids and alcohols, and the breakdown of proteins to amino acids and peptides of low molecular weight. The reduction of the foods to a solution of small, readily diffusible molecules is therefore largely accomplished. In this process gastric digestion plays a minor part; in fact, digestion is adequate in the absence of the stomach. Failure of pancreatic secretion, or pathological deficiency in pancreatic enzymes, results in serious losses of carbohydrate, nitrogen, and fat in the feces (Table 37).[19] The data illustrate the importance of the pancreatic enzymes in digestion.

TABLE 37

THE EFFECTS OF ORAL ADMINISTRATION OF PANCREATIC JUICE ON THE UTILIZATION OF INGESTED FAT AND NITROGEN IN PATIENTS WITH DEFICIENT PANCREATIC ENZYMES

Patients	Daily intake, g.		Wet weight of feces, g.	Fecal nitrogen, g.	Ingested nitrogen, %	Fecal fat, g.	Ingested fat, %
	Fat	Nitrogen					
Untreated	112	10.3	441	7.84	76	74.4	66
Treated	112	13.28	218	2.99	22.5	27.3	24.4

5. The Intestinal Secretions[2]

(a) *Nature and Functions*

The glands of the intestinal mucosa produce a clear, alkaline, mucous secretion (the *succus entericus*) of limited digestive powers. Throughout most of the small intestine the glands are simple in type, and secrete mainly in response to local stimulation. In the upper duodenum, the glands are larger and more profuse, and they secrete copiously in response to acid, salts, secretagogues, and secretin itself. These glands, first described by Brunner, are continuous with the glands of the pyloric region of the stomach (which also produce an alkaline secretion), and they share with the pyloric glands the task of neutralizing the acid chyme. Since the rapid neutralization of acid is a prerequisite of pancreatic digestion, the secretion of Brunner's glands is coordinated with the pancreatic secretion by their response to the same agents that stimulate the pancreas. There is thus a minimum delay in the onset of intestinal digestion.

---

[19] J. M. Beazell, C. R. Schmidt, and A. C. Ivy, *J. Am. Med. Assoc.*, **116**, 2735 (1941).

[20] H. W. Florey, R. D. Wright, and M. A. Jennings, *Physiol. Revs.*, **21**, 36 (1941).

The functions of the intestinal juice are the protection of the mucosa against mechanical and chemical damage, the neutralization of acid, the provision of the activator, enterokinase, and the supply of fluid (as much as 3 liters per day) for the solution and transport of the products of digestion.

(*b*) *The Digestive Properties of the Succus Entericus*

Many enzymes have been reported in the intestinal juice—phosphatase, maltase, sucrase, lactase, and numerous peptidases—and it is generally considered that these enzymes carry out the last simple stages of digestion in the intestine. The purest samples of the juice, however, free of cells and cell debris, usually contain only enterokinase and amylase (which may come from the blood or the pancreas). This implies that the enzymes usually ascribed to the *succus entericus* are intracellular enzymes, and that the intestinal juice normally contains only one proper enzyme, enterokinase, which is responsible for the activation of trypsinogen.

If this is correct, many of the final stages of digestion—the hydrolysis of disaccharides, the splitting of small peptides to amino acids, and the breakdown of simple extractives like phosphate esters and nucleotides—probably take place within the cells of the intestinal mucosa. There are no technical obstacles to this. After pancreatic digestion, most of the large molecules of the foodstuffs have been simplified and dissolved. The relatively simple extractives need only be set free from the tissues. All of these digestive products may easily diffuse into the intestinal cells, where they can be broken down. In these circumstances, the final stages of digestion of many substances may be concurrent with the first stages of their metabolism, since within the cells there is an extensive opportunity for resynthesis, rearrangement, and specialized distribution. Very few details of these processes are known. That they occur, and may be extensive, is indicated by studies with "tagged" atoms and molecules, in which the intestinal mucosa is found to be among the most active tissues involved in the dynamic interchange of the body constituents.[21]

(*c*) *The Enzymes of the Intestinal Mucosa*

Active preparations of the enzymes usually ascribed to the *succus entericus* are made by extracting the intestinal mucosa, a fact which supports the view that these are truly intracellular enzymes. Their investigation has been seriously neglected, possibly because of technical difficulties, but more likely because the implications of the absence of enzymes from intestinal juice have not yet been widely appreciated. The best known of the intestinal enzymes are the peptidases, in which interest has been revived by the development of new methods of study.[22]

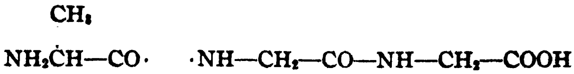
---

[21] Rudolf Schoenheimer, *The Dynamic State of the Body Constituents.* Harvard Univ. Press, Cambridge, 1942.

[22] M. J. Johnson and Julius Berger, *Advances in Enzymology,* Vol. 2. Interscience, New York, 1942, p. 69.

The peptide-splitting enzymes of the intestinal mucosa were called "erepsin" by their discoverer, Otto Cohnheim. It is now known that erepsin is a mixture of peptidases, which may be differentiated by their specificity toward synthetic substrates. At present only two have been isolated, purified, and characterized:

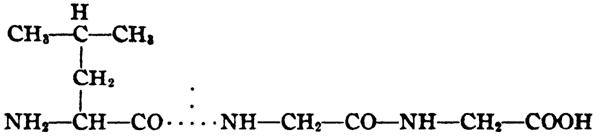
1. Aminopolypeptidase, which attacks peptides of the constitution of alanyldiglycine:



$$\overset{CH_3}{NH_2\dot{C}H}\text{—}CO\cdot\quad\cdot NH\text{—}CH_2\text{—}CO\text{—}NH\text{—}CH_2\text{—}COOH$$

The enzyme can split dipeptides, but acts more rapidly on polypeptides. There must be a hydrogen atom on the nitrogen atom of the hydrolyzed bond. Aminopeptidase attacks the peptide bond at the amino end of the chain, and the free amino group is essential. The specificity of this enzyme is broader than that of most; nevertheless it appears to be a single individual.

2. Leucine-aminopeptidase, which splits substrates of the type of leucyldiglycine:



It attacks di- and polypeptides, if (*a*) there is a leucine side chain at the amino end of the peptide chain; (*b*) the amino group of leucine is unsubstituted; (*c*) there is a hydrogen atom on the nitrogen atom of the hydrolyzed bond. Magnesium or manganese ions activate this enzyme, which is rather unusual for catalysts of this type.

The study and identification of the dipeptidases of the intestinal mucosa await the further development of experimental methods.

6. The Significance of the Gastrointestinal Peptidases

At present the fate of only a few of the 22 or more amino acids making up proteins can be discussed in terms of the enzymatic reactions in which they are engaged during digestion. The exploration of the pancreatic and intestinal enzymes has only just begun, but it has already raised a number of interesting questions. The high order of specificity of the peptidases indicates that the digestion of protein, although it is largely extracellular, follows a definite pattern. The repetition of this pattern of specificities by the enzymes of many species of animals suggests that, like the recurrence of combinations in ciphers, there is a general pattern of chemical structures recurring with relatively high frequency in the constitution of most proteins. Since the system of gastrointestinal peptidases is an effective barrier against the entrance into the circulation of amino acid combinations foreign and harmful to the organism it is posble that their specificities are directed toward the resolution of those structures with the greatest economy of effort. It may be that defects in the system are responsible for the extraordinary sensitivity toward certain foods exhibited by some individuals. In this respect, the peptidases of the intestinal mucosa may be particularly important, since their substrates are readily diffusible products of protein breakdown, easily capable of entering the tissues.

Since the size of substrate molecules is not the determining factor, the action of the peptidases in the intestine is not necessarily successive and independent, but is more likely continuous and interdependent within broad limits. An example of this is the "co-substrate effect" described by Behrens and Bergmann.[23] Glycylleucine is not hydrolyzed by papain unless acetylphenylalanylglycine is also present. The reaction takes place in three steps. First, the two dipeptides are combined to form the tetrapeptide, acetylphenylalanylglycylglycylleucine; second, leucine is split off from the tetrapeptide; and, third, one glycine residue is split off from the remainder, leaving the "co-substrate," acetylphenylalanylglycine, intact. Because the equilibrium points are far on the side of hydrolysis, the amount of tetrapeptide formed is very small, but its rapid removal in the two subsequent hydrolyses permits an appreciable total synthesis of the essential intermediate. If reactions of this type can occur in the intestine, a limited number of peptidases of restricted specificity, by working together, may be able to effect a fairly complete hydrolysis of many different types of protein. This appears to relax one awkward requirement of the hypothesis of chemical specificity of the peptidases—that there be an almost unlimited number of enzymes to deal with all the combinations of amino acids present in proteins. The number of peptidases is undoubtedly large, but it may be limited so long as one requirement in addition to the production of soluble and diffusible molecules is fulfilled: that the structures essential to the behavior of proteins as foreign bodies be destroyed during digestion.

The growing evidence that the peptidases of the intestinal mucosa are true intracellular enzymes reopens the question whether peptides as well as amino acids are absorbed from the intestine. Since the intracellular peptidases can be coupled with energy-yielding systems, they may be as much concerned with the synthesis of peptides as with their hydrolysis, and they may therefore play an important part in determining the form in which protein nitrogen is transported from the intestine to the tissues.

7. The Secretion, Composition, and Functions of the Bile[24]

Bile is formed in the liver and stored in a concentrated form in the gall bladder. The presence of acids, fats, and the products of gastric digestion in the intestine causes contraction of the gall bladder and discharge of the bile into the duodenum through the common bile duct. The gall bladder contracts in response to a hormone, *cholecystokinin*, liberated from the intestinal mucosa by the secretagogues.[25] Thus the secretions of the bile, the pancreatic juice, and the *succus entericus* are all coordinated by similar humoral mechanisms. The continued secretion of bile during digestion is assured by the bile acids, which are themselves the most powerful of cholagogues. The bile is reabsorbed, along with the intestinal contents. It is returned to the liver, which may re-form and re-secrete it. There is thus an economical interior circulation of the bile.

---

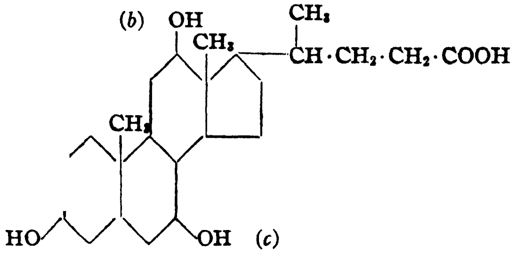
[23] O. K. Behrens and Max Bergmann, *J. Biol. Chem.*, **129**, 587 (1939).

[24] C. L. A. Schmidt, *Physiol. Revs.*, **7**, 129 (1927).

[25] A. C. Ivy, *Glandular Therapy and Physiology.* Am. Med. Assoc., Chicago, 1942; Chap. XXX, p. 523.

Bile is a mucous, alkaline (*p*H 7–8), green or greenish-yellow fluid. Its major components are the salts of the bile acids, the bile pigments, lecithin, cholesterol, neutral fat, and inorganic salts, particularly of iron, copper, calcium, and magnesium. Bile is an excretion as well as a secretion, and it is not yet entirely clear which of its constituents are essential to its digestive function. The bile pigments, derived from the breakdown of the porphyrin nucleus of hemoglobin, are purely excretory products.

The digestive properties of bile appear to be due mainly to the bile salts, sodium glycocholate and sodium taurocholate, formed by the conjugation of glycine and of taurine (aminoethylsulfonic acid, $NH_2—CH_2—CH_2—SO_3H$) with the cholesterol derivative, cholic acid:



(There are several derivatives of cholic acid in bile: desoxycholic acid, lacking a hydroxyl group at (*c*); chenodesoxycholic acid, lacking a hydroxyl group at (*b*); and lithocholic acid, lacking hydroxyl groups at (*b*) and (*c*). Their proportions differ in the bile of different species, but it is not known if this is significant in digestion.)

The bile salts lower the surface tension of their solutions and, acting with fatty acids and cholesterol, assist in the emulsification of the lipids, promoting their hydrolysis and possibly their absorption. They are also, because of their polar nature, good hydrotropic agents, forming loosely organized polymolecular complexes with fatty acids to facilitate their absorption by the intestinal epithelium. Since some fatty acids, like oleic acid, are themselves good lipid solvents, their water-soluble complexes with the bile salts may form the vehicle by which many of the lipids can be dissolved and absorbed.

The discharge of bile by the brisk contraction of the gall bladder makes possible an immediate response to the presence of lipids in the intestine. After removal of the gall bladder, the liver secretes bile constantly into the intestine. Since there is no longer a stored reserve of bile, this secretion may not be enough to meet the requirements of a fatty meal. More often, however, the secretion is in excess of the requirements of a nonfatty meal, especially when excessive amounts of cholagogues are carelessly taken in the meal. There may then be regurgitation of bile, and consequent gastric distress, during digestion.

V. ABSORPTION[26]

1. The Major Factors in Absorption

The average daily intake of dry food solids is about 600–700 g. Water in the food and in beverages amounts to 1–3 liters. The total daily secretion of digestive fluids may be more than 8 liters, or nearly three times the volume of the blood plasma from which the fluid is drawn. Only about 400 g. of a watery residue of these materials pass into the large intestine; the rest is absorbed in the small intestine. Since the volume of the digestive secretions required for a single meal may nearly equal the blood plasma volume, secretion and absorption must keep in good pace if serious circulatory disturbances are to be avoided. The volume of the intestinal contents is largely determined by their concentration, since the intestine tends to maintain the osmotic pressure of the chyme at levels nearly isotonic with the blood. The rapid absorption of solutes is a requirement of the rapid return of fluid to the circulation. The processes of digestion, secretion, and absorption must therefore be concurrent, and nicely balanced against one another.

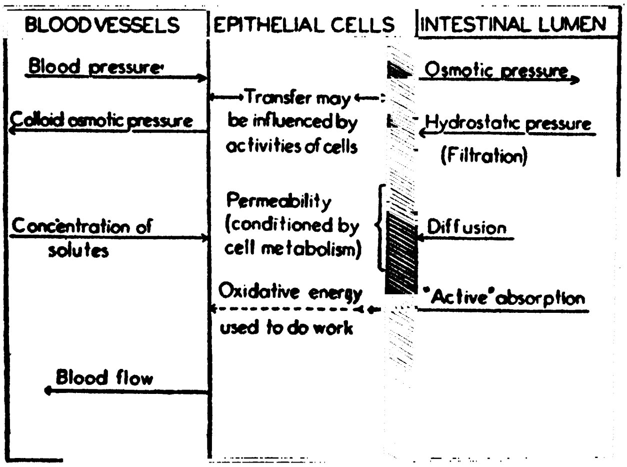


Fig. 25.—Simple scheme showing the major forces concerned in absorption from the intestine. The arrows indicate the direction of movement of fluids and solutes under the influence of the indicated forces.

If enough time is allowed, and if the concentration is high, soluble substances may diffuse from any part of the alimentary tract, but the small intestine is especially adapted for absorption. It is the longest part of the alimentary canal (about 10 ft.).

---

[26] F. Verzár and E. J. McDougall, *Absorption from the Intestine*. Longmans, Green, London, 1936.

The intestinal epithelium has many folds and ridges, and is densely supplied with millions of tiny, fingerlike projections, the *villi*, which greatly increase the surface of the mucosa. The surface of the human small intestine is estimated at about 10.5 square meters, as against 1.3 square meters for a cylinder of the same dimensions. The epithelial cells are of a specialized type usually seen where active absorption and secretion take place. Beneath the single layer of epithelial cells is a network of blood capillaries with a total surface at least as great as the surface of the epithelium. An additional large surface is presented by the lymphatic channels taking origin in the villi. Finally, the moving blood stream, itself a tremendous, constantly renewed surface, rapidly carries away the substances diffusing from the epithelial cells.

The balance of the major forces concerned in the absorption of water and solutes from the intestine is outlined in Fig. 25. The fundamental physical forces involved in absorption are osmosis and diffusion, but the movements of fluids and solids may be modified by the epithelial cells, which can expend oxidative energy to do work against concentration gradients, and to maintain the characteristic organization of their membranes. This is indicated by the fact that even during the absorption of a salt solution the rate of blood flow, the oxygen consumption, and the carbon dioxide production in the intestine may increase by more than 150%. The great area of the intestinal epithelium, and the fact that the circulation of the blood, by removing absorbed substances, prevents or delays the establishment of equilibria between the different parts of the system, suggest that diffusion alone might account for the absorption of most substances from the intestine. But in some instances the active absorption of solutes by the epithelial cells may be necessary to maintain a proper balance between the blood volume and the volume of fluid in the intestine.

The questions asked in the study of absorption are: (*a*) the form in which the substance is absorbed; (*b*) whether absorption is a passive or an active process; (*c*) the mechanism of the active process; and (*d*) the influence of other substances on absorption. Partial answers to these questions have been obtained for many of the major components of the foods. Almost all of the work has been done on dogs, cats, rabbits, and rats. The experimental surgeons of the 19th century followed the admirable course of studying the absorption of whole natural meals in dogs with intestinal fistulas, but their chemical methods were not adequate to the task. In recent years, the absorption of single substances from the intestine of small animals has been widely studied, but this method yields no information about the behavior of these substances in the presence of all the components of the normal diet, during digestion. Very few adequate studies have been made on absorption from the human small intestine. The development of the method of intestinal intubation, using the multiple-lumen Miller-Abbott tube,[27] has only recently made possible the direct study of absorption in the human subject.

A large functional reserve and a ready adaptability to changing conditions are outstanding characteristics of the intestine. In man, the whole of the daily requirement of ascorbic acid may be absorbed from a 0.1% solution in 1 hr. by only 90 cm. of small intestine.[28] The absorption, over a period of years, of as much as 70% of the calories ingested has been observed in a subject with only 3 ft. of small intestine remaining after an operation.[29] The rapid accommodation of the small intestine to diets of unusual composition has often been observed. On diets very high in carbohydrate or in protein there may be a considerable increase in the rate of absorption of these substances. This is partly a consequence of the physical enlargement of the intestine, particularly in animals with unrestricted appetite, but in addition there seems to be an increase in the enzymes or enzyme systems responsible for the digestion and absorption of the major component of the diet. Conversely, inanition, even for brief periods, may cause a marked decrease in the rate of absorption of substances from the intestine. This is important, since changes in absorption rates due merely to loss of appetite or to prolonged fasting may be mistakenly attributed to some experimental measure, for example, the removal of an endocrine gland.[30]

---

[27] W. O. Abbott, *Advances in Internal Medicine*, Vol. 1. Interscience, New York, 1942, p. 1.

2. The Absorption of Individual Substances

(a) *Water and Salts*

The movement of water to and from the intestine is controlled largely by osmotic forces.[31] Water is freely absorbed from hypotonic solutions while hypertonic solutions are diluted by the movement of water into the intestine. Thus the movements of water and of solutes are independent, but the *direction* of movement of water is dependent on the concentration of solutes, and is determined by the rate of production of soluble substances in digestion, and by their rate of diffusion and absorption. The action of purgatives like Epsom salts is due in part to the fact that they are only slowly absorbed and therefore detain water in the intestine. The administration of concentrated solutions of very soluble substances, as for instance 30–50% glucose, or solutions of amino acids or protein hydrolyzates, often causes diarrhea, since they not only draw water into the intestine, but also excite marked peristalsis and reduce the time of passage of material through the intestine. The incautious feeding of predigested foods, particularly of protein digests, may therefore be both awkward and uneconomical. In the small intestine, hydrostatic pressure and filtration are unimportant, since small pressures are developed only locally in the course of peristaltic contractions. In the large intestine, however, hydrostatic pressures up to 400 mm. of water may be developed and maintained by the tonic contraction of the ileocecal valve and the anus. Under this pressure, water and dissolved substances may be removed by filtration during the formation of the feces.

The problems of the absorption of individual salts (or ions) are largely unsettled.

---

[28] J. T. L. Nicholson and F. W. Chornock, *J. Clin. Investigation*, **21**, 505 (1942).

[29] W. R. Todd, M. Dittebrandt, J. R. Montague, and E. S. West, *Am. J. Digestive Diseases Nutrition*, **7**, 295 (1940).

[30] Rose Marrazzi, *Am. J. Physiol.*, **131**, 36 (1940).

[31] S. Goldschmidt, *Physiol. Revs.*, **1**, 421 (1921).

Since they may differ widely in their absorption rates, some salts must be actively absorbed by the mucosa. The problem is complicated by the secretion or excretion of several salts by the intestine. Practically, the question is not whether inorganic solutes are absorbed by diffusion or by some active process, but whether conditions in the digestive tract limit the availability of some substances for absorption. Calcium or magnesium, for instance, must be present as soluble salts. The formation of their insoluble phosphates, due to an excess of phosphate or to the alkalinity of the intestinal contents, prevents their absorption. The absorption of calcium is facilitated by moderate amounts of fat, but fats or fatty acids in excess, or a failure of fat absorption (in biliary obstruction) may cause large losses of calcium in the feces. Many of these relationships remain to be worked out, but it is important to remember that the absorption of some inorganic ions is probably closely related to the digestion and absorption of the organic constituents of the diet.

(*b*) *Carbohydrates*

The carbohydrates most commonly present in the chyme during digestion are glucose and the disaccharides maltose (from starch), sucrose, and lactose. It is generally taught that the disaccharides are hydrolyzed by specific enzymes in the intestinal juice, so that the monosaccharides glucose, galactose, and fructose are the sugars absorbed by the intestine. Since the presence of invertase, maltase, and lactase in the *succus entericus* may now be considered doubtful, the question whether the disaccharides are absorbed before or after hydrolysis is an open one. It has occasionally been observed that maltose and sucrose are more rapidly absorbed than they are hydrolyzed, but since neither sugar normally appears in the blood, their hydrolysis in the intestinal cells must be rapid and complete. Lactose is slowly absorbed, and when fed in large amounts it may be fermented to lactic acid by bacteria in the intestine. This circumstance has been used to produce a more acid intestinal reaction in order to facilitate the absorption of calcium. Since the predominant carbohydrate in the human diet is starch, which yields maltose in digestion, the absorption of this disaccharide requires more careful study. It is possible, in view of the rapidity with which it leaves the intestine, that maltose, like glucose, is absorbed by an active process. In any event, the hydrolysis of disaccharides within the intestinal cells is bound to increase their absorption rates by maintaining a steep concentration gradient.

The monosaccharides *glucose, galactose,* and *fructose* are more rapidly absorbed from the intestine than mannose or sorbose, the pentoses, and the much smaller molecules of inorganic salts (Table 38). Their absorption rates are practically independent of their concentration in the intestine (up to about 13%). When more than one of them is present, the total amount of sugar absorbed is the same as if only one were present (that is, they seem to share a common mechanism of fixed capacity). The level of sugar in the blood is without effect on their absorption rates. These characteristics of the absorption of glucose, galactose, and fructose are abolished by killing the intestinal mucosa with hot water, or by the cellular poisons cyanide, iodoacetic acid, or phlorizin. The intestinal cells therefore intervene actively in the absorption of these sugars.

Because iodoacetic acid and phlorizin both interrupt phosphorylation processes, Verzár has concluded that the actively absorbed sugars are phosphorylated in the intestinal cells, and it is now generally agreed that the formation of hexose phosphates is an integral part of the absorption mechanism. But it is probable that this is a single step in a more complex process. Since it is now known that the utilization of energy in biological systems involves the formation and transfer of phosphate in special organic combinations,[82] iodoacetic acid and phlorizin may inhibit more fundamental processes than the formation of hexose phosphate. Fructose is readily converted into glucose in the intestinal cells by steps involving its rapid preliminary phosphorylation. If phosphorylation alone were responsible for the active absorption of sugars, it is difficult to see why fructose is more slowly absorbed than glucose or galactose.

TABLE 38

RELATIVE RATES OF ABSORPTION OF SUGARS IN THE RAT INTESTINE

(Glucose = 100%)

Glucose	100	Sorbose	30
Galactose	115	Xylose	30
Fructose	44	Arabinose	29
Mannose	33	Rhamnose	29

The provisions for the rapid absorption of carbohydrate emphasize its importance to the organism as a readily available source of energy. The absorbed carbohydrate leaves the intestine mainly in the blood, and is distributed to the tissues. Part of it is oxidized and the remainder is stored as glycogen in the liver and the muscles, or, when these stores are filled, it may be converted into fat.

(c) *Lipids*

There are two current views concerning the absorption of neutral fats. Verzár has proposed that fats must be completely hydrolyzed to fatty acids and glycerol in the intestine. Glycerol is readily absorbed, and the fatty acids, by forming water-soluble complexes with the bile salts, can also be absorbed. In the absence of bile or of lipase there is complete failure of fat absorption. In the intestinal cells neutral fat is actively resynthesized by a process in which phospholipids are intermediates. Iodoacetic acid and phlorizin inhibit fat absorption by interrupting the phosphorylation processes upon which the resynthesis of neutral fat depends. Most of the newly formed fat, together with some phospholipid and cholesterol esters, passes into the lymphatic vessels and is propelled by the pumping action of the villi to the thoracic duct and finally to the blood in the jugular vein. This sequence of events is deduced in part from histological studies with differential stains, which show an accumulation of free fatty acids in the intestinal cells in the early stages of absorption and of neutral fat in the later stages. When the cells are poisoned, only free fatty acids accumulate in large amounts. Other experimental evidence agrees with this hypothesis in principle. During fat absorption there is a rapid turnover of phospholipid phosphorus in the intestinal mucosa. Labeled fatty acids are readily incorporated into phospholipids, indicating at least a partial resynthesis of these compounds. The feeding of free fatty acids results in the appearance, in blood and lymph, of neutral fat, phospholipids, and cholesterol esters, showing that syntheses do in fact take place in the intestinal mucosa.

---

[82] F. Lipmann, *Advances in Enzymology*, Vol. 1. Interscience, New York, 1941, p. 99.

The alternate hypothesis of Frazer[33] denies that hydrolysis of neutral fat is essential to its absorption. He observes that when Sudan III-stained neutral fat is fed to an animal, there is an increase in chylomicrons[34] in the systemic blood, and the stained fat is found mainly in the peripheral fat depots. When stained fatty acids are fed, chylomicrons increase in the portal blood, and the fat formed during absorption is found mainly in the liver. Hence the routes of distribution of preformed and of newly synthesized fat are different, and the traditional view that the bulk of absorbed fat is transported to the systemic blood by the lymphatics is not invariably correct. Since the transport of fat across cell membranes in the body does not require hydrolysis and resynthesis, there is no obvious reason why neutral fat cannot also enter the intestinal cells. The conditions under which this may occur have been neatly demonstrated by Frazer and his colleagues.[35] If enough oleic acid and cholesterol are added to an aqueous suspension of paraffin, a stable fine emulsion (particle size 0.5 $m\mu$) is formed. As much as 60% of the administered hydrocarbon may be absorbed from such an emulsion by the small intestine of the rat. Olive oil spontaneously forms a fine emulsion of this type in the rat intestine, where adequate cholesterol is provided in the bile, and fatty acid is available from the partial hydrolysis of fat. In these circumstances, the remaining triglycerides, like the paraffin, may be directly absorbed.

Frazer's hypothesis satisfactorily explains why fat absorption is adequate in some animals in which the amounts of lipase and of bile salts secreted are too small to account for complete hydrolysis and solution according to Verzár's views. The hypotheses are not incompatible, but are complementary, and they may be taken together to give a more complete account of fat absorption.

The neutral fats and fatty acids facilitate the absorption of calcium and phosphorus and provide a vehicle for the absorption of fat-soluble substances: vitamins, carotenoids, sterols, and steroid derivatives. The intestine discriminates, however, between cholesterol and certain plant sterols—the latter are not absorbed.

Most of the absorbed fat is transported by the lymphatic system to the systemic blood and is distributed to the fat depots. Some fat, mainly phospholipids and cholesterol esters, goes directly to the liver. Although fat is the main storage form of food energy in the body, the stores are not at all static. There is a constant turnover of depot fat in which the resynthesis and revision of fatty acids and triglycerides, their withdrawal for oxidation, and replacement by fat synthesized from carbohydrate and protein all take part.[36] These processes, coupled with the partial resynthesis and special distribution of dietary fat during digestion and absorption, tend to maintain a characteristic composition of the body fat, except when the composition of the fats fed is made widely different from that of the normal diet.

---

[33] A. C. Frazer, *Physiol. Revs.*, **20**, 561 (1940).

[34] "Chylomicrons" is the term applied to the small bright particles seen in blood when it is viewed in the dark-field microscope. These particles are believed to be fine globules of fat surrounded by a protein monolayer. Their numbers correspond closely with the fat in the blood as determined by chemical methods, and the rise and fall of the chylomicron count in the blood faithfully mirrors the progress of digestion and absorption of fat after a meal.

[35] A. C. Frazer, H. C. Stewart, and J. H. Schulman, *Nature*, **149**, 167 (1942).

(*d*) *Products of Protein Digestion*

Since the intestinal peptidases appear to be intracellular enzymes, the mixture of amino acids and polypeptides remaining after pancreatic digestion probably represents the forms in which protein is absorbed in the intestine. Further breakdown and resynthesis of these substances in the intestinal cells may facilitate their absorption. The fact that the absorption rates of amino acids in the rat range from 0.14 to 0.84 g. per 100 g. of rat per hour suggests that there are specific mechanisms for the active absorption of some amino acids or that certain amino acids are more readily engaged in synthetic processes in the cells than others. When the variety and chemical specificities of the intestinal peptidases are better known, it may be possible to correlate their properties with the absorption rates of the amino acids with which they are concerned.

During absorption there is an increase in the blood amino nitrogen, particularly in the portal vein, indicating that most of the absorbed nitrogen passes directly to the liver. Both amino acids and polypeptides (either directly absorbed or synthesized in the intestinal cells) may be present in the blood, but there are no reliable estimates of their relative proportions, so that at present the importance of polypeptides in the transport of nitrogen cannot be assessed.

The amino acids of dietary protein are widely distributed and follow many metabolic pathways in the body. 1. A portion of them is deaminated to form ammonia and keto acids. (*a*) The ammonia is converted to urea in the liver, and excreted; it is excreted directly by the kidney, to conserve base, or it is used in the synthesis of new amino acids. (*b*) The keto acids are oxidized to provide energy, or used in the synthesis of other amino acids, carbohydrate, or fat. 2. Large proportions of ingested amino acids are incorporated in body protein. The speed and extent of this process have only recently been demonstrated by the work of Schoenheimer and his colleagues with the heavy isotopes of nitrogen and hydrogen.[36] In an adult animal in nitrogen balance as much as 50% of the dietary nitrogen may replace body nitrogen in continuing processes of breakdown and resynthesis of tissue protein. 3. Certain amino acids are required in the synthesis of special substances, *e. g.*, tyrosine or phenylalanine for thyroxine and epinephrine; glycine, arginine, and methionine for the synthesis of creatine. 4. Toxic substances may be conjugated with glycine, cystine, or glutamine to facilitate their removal from the body. No single amino acid follows an exclusive course; each appears to be involved in all the reactions open to it.

---

[36] Rudolf Schoenheimer, *The Dynamic State of the Body Constituents.* Harvard Univ. Press, Cambridge, 1942.

The absorption of intact protein in the intestine does not normally take place. Individuals are found, however, who exhibit allergic sensitivity to certain foodstuffs. This phenomenon often occurs in the very young, and it is relatively easy to induce experimentally in young animals by feeding large amounts of a foreign protein, like egg albumin. Special sensitivity to foodstuffs appears to be due to the absorption of intact protein, but it has not been decided whether in these instances the intestinal mucosa is unusually permeable to protein, whether the mucosa is especially liable to damage which permits direct access of the intestinal contents to the blood stream, or whether there are deficiencies in the system of gastrointestinal peptidases which prevent the destruction of amino acid combinations which can give rise to immune reactions in the body.

(*e*) *The Influence of Endocrines on Absorption*

Verzár and his colleagues have reported that the selective absorption of carbohydrate and the resynthesis of neutral fat during absorption are almost completely abolished after adrenalectomy in the rat. Since these effects are similar to the effects of iodoacetic acid and phlorizin, they have concluded that the adrenal cortical hormone is essential for phosphorylation processes, and that a failure of phosphorylation, particularly of riboflavin, is responsible for the failure of absorption in adrenalectomized animals. Numerous attempts to confirm this hypothesis have failed. It is generally found that if adrenalectomized animals are maintained in good condition with extra sodium chloride in their drinking water, the rates of absorption of carbohydrate and fat are almost normal. There are no apparent abnormalities in the behavior of riboflavin in adrenalectomized animals, and the phosphorylated vitamin does not prolong life after adrenalectomy. It appears probable that the failure of absorption in untreated adrenalectomized animals is due to secondary rather than to primary effects of severe adrenal insufficiency.

Similar defects in the absorption of carbohydrate in hypophysectomized animals have been ascribed to the atrophy of the adrenal cortex which occurs after hypophysectomy. It is more likely that the reduced absorption rates are due to atrophy of the thyroid gland. Thyroidectomy results in a marked reduction in the absorption rates of carbohydrate and fat in the rat, while hyperthyroidism causes a large increase in the rates of absorption of the actively absorbed sugars and fatty acids.[37] Since the endocrine glands have profound effects upon metabolism, it is expected that they should influence the complex functions of the alimentary canal, but at present the thyroid hormone is the only one which has been definitely shown to influence intestinal absorption.

VI. THE FUNCTIONS OF THE LARGE INTESTINE

In the large intestine the final stages of the absorption of water and solutes take place, and the residual materials are made into feces, accumulated in the rectum, and finally excreted. The dilute watery residue that is passed into the large intestine contains small amounts of carbohydrate, fat, protein, and salts, together with the indigestible material, of which the chief representative is cellulose, that is classed as "roughage" in the diet. These substances are subject to intense bacterial action in the large intestine, which has a large and variegated bacterial population. The chief products of bacterial fermentation and putrefaction (see pp. 143–146) are as follows.

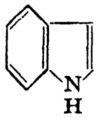
---

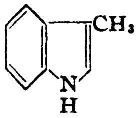
[37] T. L. Althausen and M. Stockholm, *Am. J. Physiol.*, 123, 577 (1938).

Gases.—Carbon dioxide, ammonia, hydrogen sulfide, methylamine, hydrogen, and methane make up the intestinal flatus. From 500 to 600 ml. per day have been collected from normal human subjects.

Acids.—Acetic, lactic, and butyric acids are formed by the fermentation of carbohydrates and by the reductive deamination of amino acids.

Amines.—Amines are formed by the decarboxylation of amino acids. The more important of these are tyramine, from tyrosine; histamine, from histidine; and putrescine and cadaverine (so-called "ptomaines") from arginine and lysine, respectively. More complex products of decarboxylation are indole and skatole, which are derived from tryptophane:


Indole


Skatole

These substances are partly responsible for the odor of the feces. Many of the amines are highly toxic, and they have been held responsible for the symptoms of "autointoxication" arising in constipation. Since the symptoms may be evoked simply by stuffing the rectum with cotton, there is reason for thinking that normally the liver is fully capable of detoxifying the absorbed products of bacterial decomposition, and that true intoxication of intestinal origin occurs only in exceptional cases.

Phenol.—Phenol may be formed by the decomposition of tyrosine.

Cystine.—Cystine (and possibly methionine) may be degraded to mercaptans ($CH_3.SH$—methylmercaptan), highly odorous compounds, which can in turn give rise to methane and hydrogen sulfide.

Bile Pigments.—The bile pigments are degraded to products (urobilin and stercobilin) which are responsible for the characteristic color of the feces.

These products may be absorbed during the removal of water under pressure in the large intestine. Useful materials are thus recovered, and waste substances are detoxified by the liver and excreted by the kidneys. The nature of the products of bacterial action is determined by the diet, since the continued growth of the intestinal flora depends upon the substrates presented to them. A high carbohydrate diet favors the development of acid-producing bacteria, while diets high in protein encourage the growth of bacteria responsible for the production of toxic decomposition products of amino acids.

About 25% of the dry weight of the feces is made up of bacteria. The remainder consists of materials which have escaped digestion and absorption, substances excreted by the intestine, and cellular debris from the intestinal mucosa. The bulk of the feces depends upon the amount of roughage in the diet. Since the regular evacuation of the bowel depends upon stimuli arising from the accumulation of feces in the rectum, the inclusion in the diet of moderate amounts of indigestible material (*i. e.*, plant fiber or bran) is often recommended as a means of preventing or alleviating constipation.

Selected Bibliography

Beaumont, W., *Experiments and Observations on the Gastric Juice and the Physiology of Digestion.* Peter Smith, New York, 1941.

Best, C. H., and Taylor, N. B., *Physiological Basis of Medical Practice.* Wood, Baltimore, 1940.

Ivy, A. C., *Glandular Therapy and Physiology.* Am. Med. Assoc., Chicago, 1942.

Pavlov, I. P., *The Work of the Digestive Glands.* Griffin, London, 1910.

Schoenheimer, R., *The Dynamic State of the Body Constituents.* Harvard Univ. Press, Cambridge, 1942.

Verzár, F., and McDougall, E. J., *Absorption from the Intestine.* Longmans, Green, London, 1936.

Wolf, S., and Wolff, H. G., *Human Gastric Function.* Oxford Univ. Press, New York, 1943.

Chapter XI

FOOD SPOILAGE AND FOOD POISONING

H. Orin Halvorson
Minneapolis, Minnesota

I. INTRODUCTION

The preservation of food, or the prevention of food spoilage, has always been one of the major problems of human civilization. From the earliest dawn of human existence up to the present complex social structure, man has always been concerned with means of preserving his food.

The very laws of nature make this an inescapable problem. Whenever a plant or animal dies, nature sees to it that the organic material involved is broken down or is decomposed. This allows for the return of the elements into simple compounds that can again be used by plants or animals. Were this not the case, all the carbon and nitrogen would soon be tied up in complex forms which could no longer be utilized. This would eventually lead to the disappearance of both plant and animal life from this earth. It is for this reason that nature has provided a means for the decomposition of every organic molecule that is produced in nature. The person who wishes to prevent food spoilage is, therefore, working counter to these forces of nature, and this makes food preservation a difficult and involved problem.

Man's experience with food spoilage is as old as man himself. Most types of spoilage that we can observe today have occurred since before the days of recorded history. It is natural, therefore, that some of the common methods of food preservation have been handed down to us from antiquity. Even though the human race has experienced food spoilage from the earliest times, ideas of what constitutes spoilage have been undergoing continual change. Changes in food that may have been regarded as normal and desirable in the past may be classified as spoilage today. Before the day of the icebox, people did not regard the souring of milk as undesirable. In many cases milk was not considered good for food until it had soured. Today, however, most people look with disdain upon sour milk and consider it spoiled.

Since we are continually changing our concepts of what constitutes wholesome food, it is difficult to define spoilage. It does not necessarily mean that the food has become unwholesome. Any change that makes food undesirable for a given population for aesthetic or health reasons must be considered as spoilage. It is apparent from this definition that food may be regarded as spoiled from the standpoint of health, when it would appear to be good as far as aesthetic values are concerned. Likewise the aesthetic value of the food may have undergone tremendous changes without altering its nutritive value. It is also true that what constitutes spoilage for one population may not be considered so by another. For example, there are certain kinds of cheese that one group of people would regard as spoiled and another group would consider as particularly choice.

In spite of the fact that individuals may not be able to agree on what constitutes food spoilage, all of them are generally afraid to eat what they consider spoiled food. The reason for this is that the average individual does not know how to differentiate those changes that affect the nutritive values from those that have to do only with aesthetic values. For example, the general public is well trained not to use the product from a can that is bulging. The contents of such a can may not be dangerous, but there is no way the consumer can determine this.

The ordinary and common types of food spoilage are caused by changes of a biological nature. Food may also be spoiled by the introduction of undesirable chemicals. Thus the addition of arsenic or mercuric chloride would certainly spoil the food. In this chapter, however, I shall deal only with the biological changes. This will involve changes attributed to the growth of bacteria, yeast, and molds, changes caused by enzymes, and, in some cases, changes resulting from oxidation.

II. BIOCHEMICAL FACTORS CAUSING SPOILAGE

In most cases of food spoilage, microorganisms are responsible. Not infrequently, however, spoilage may be caused by the action of enzymes in the absence of microbial growth. In a few instances, atmospheric oxidation may be involved. Any of these factors may be acting alone or in combination with the others.

1. Carbohydrates

(a) *Production of Acid*

The changes produced by the growth of microorganisms will vary with the type or types of organisms concerned and also with the composition of the food involved. In foods that are rich in carbohydrates, bacteria will usually set up a fermentation, particularly of sugars, for these substances are more easily utilized than the proteins or fats. Although the type of breakdown of sugar differs with different microorganisms, acids are nearly always produced, particularly lactic acid. In addition, a gas is frequently liberated consisting of carbon dioxide or of a mixture of carbon dioxide and hydrogen. Other acids, in addition to lactic acid, may be formed, as well as alcohols, and traces of other compounds. If food containing sugar is contaminated by a mixed bacterial culture, as is generally the case, and is sealed in a container so as to exclude air, acids will develop to the point where they interfere with further growth of the organisms. This acid environment will depress particularly the organisms that are proteolytic in nature. As a result, the only noticeable change produced in such food will be the development of acid. The acidity will eventually become so high that it will interfere with the growth of even the acid-tolerant bacteria. The limiting acidity will vary with the sugar content, the nature of the food, and the types of organisms involved. In most cases it will be at a *p*H between 3.0 and 4.5. If the air is kept away, the acids formed will remain. In case the sugar concentration is so high that there is still sugar left as the *p*H approaches the limiting value, yeast, if present, may grow and bring about an alcoholic fermentation.

The development of acid does not render the food unwholesome, and it is not surprising that man has taken advantage of this sugar preference of microorganisms to aid him in preserving certain foods. We may sometimes wonder why people, a few generations ago, used so much sour milk. Today most milk is consumed fresh, while we regard sour milk as spoiled. This use of sour milk in past years certainly cannot be explained on the basis that people preferred it to fresh milk. A few generations ago, refrigerators were not available, with the result that milk could not be kept fresh. Man had learned through centuries that milk kept away from the air would sour and then keep for long periods of time. Thus souring was a means of preservation.

Similar methods of preservation are not uncommon in other foods. Cabbage is preserved in the form of sauerkraut; fruit juices are preserved by a combination of acid and alcoholic fermentation. In more modern days we have learned how to preserve corn for cattle by making silage. In this, as in the other cases, the sugar content is high enough to allow enough acid to be formed to limit further changes (see Vol. II, Chapter XII).

(*b*) *Fermentation and Proteolysis*

If the initial concentration of sugar is too low, the food will not be preserved by acid fermentation. In this case, the final *p*H will be high enough to allow a continued growth of the microorganisms, with a resulting destruction or neutralization of the acids that have been produced. Bacteria may do this by decomposing the acids to carbon dioxide, hydrogen, and methane, or a mixture of these gases; or by neutralizing the acids with the ammonia liberated from proteins or amino acids. The decomposition or neutralization will eventually eliminate the acid and create conditions that are favorable for the growth of proteolytic organisms. The changes thus produced will be discussed later. The lack of sufficient sugar therefore makes some foods unsuitable for preservation by fermentation alone. Thus, the farmer has difficulty in making good silage out of alfalfa, even though he always succeeds in doing so with corn.

Acids produced by fermentation will not be stable if air has free access to the food. In the presence of air the acids can be removed by oxidation through the action of molds or bacteria. Molds are much more acid-tolerant than bacteria and will grow even though the latter may be completely repressed. These molds, however, are strict aerobes and can destroy or remove the acids only in the presence of air. Bacteria that can do this are generally less vigorous. Sour milk and sauerkraut will therefore undergo further change if left open to the air. The acidity will gradually decrease and this will in turn be followed by proteolytic decomposition. A common experience for the farmer who makes silage is to find the top of the silage so decayed that it has to be discarded. The acids have been removed and proteolytic decomposition has taken place.

In the presence of sugar or acids proteolytic activity is repressed. In some cases bacteria may be able to attack both sugar and proteins. Even though they do possess this dual ability, the protein-decomposing processes are repressed in the presence of sugar. In biology this is often spoken of as the protein-sparing action of carbohydrates.[1] Bacteria that are primarily proteolytic do not grow in acid media. The net result is that foods which contain sugar will show little proteolytic change until the sugars and acids formed from them have disappeared.

The situation may be slightly different where molds are concerned. Molds may show considerable proteolytic activity even though sugars and acids are present. Thus, we find that molds growing in cheese, which is fairly acid, may attack the proteins as well as the sugar.[2] Part of the neutralization of the acid produced by their activity is brought about by the ammonia that is liberated through proteolysis.

(*c*) *Higher Carbohydrates*

Carbohydrates other than sugars may also be attacked by microorganisms. Many saccharolytic organisms produce diastase. For them, starch will be as utilizable as sugar. The rate of acid production, however, will generally be slower than from sugars because the starch has first to be hydrolyzed by the diastase. Sugar, and consequently the acids, can be produced only as fast as diastase is formed. If the latter is produced slowly, then it may be possible for proteolytic activity to take place before considerable acid is formed, and to keep pace with the acid production. Also, starch does not exhibit the protein-sparing action of the sugars. Foods rich in starch but poor in sugar cannot, therefore, be preserved as easily by fermentation.

Starchy foods may undergo other changes that contribute to spoilage. Some bacteria that can use starch as a source of energy will not only hydrolyze the starch but will convert the hydrolyzate to gums. In this way these organisms protect themselves against excessive acid productions. A rather common change of this nature is the production of ropy bread (page 713). *Bacillus mesentericus*, the causative organism, hydrolyzes the starch in the bread and converts the resulting carbohydrates to a gum. This gum has the consistency and appearance of unbaked dough. Since the sugar is removed from the sphere of action, it is not converted to acid and thus does not inhibit the growth of the organism; neither does it interfere with proteolysis. The proteins, as well as the starch, are attacked, giving rise to foul odors as well as unsightly appearance.

2. Proteins

Bacterial decomposition of proteins causes more undesirable changes in foods than the breakdown of carbohydrates. The changes involved are more complex and the end products more varied. The early stages of proteolysis involve the splitting of the protein into polypeptides, peptones, and amino acids. This is followed by a breakdown or fermentation of the latter. The microbial decomposition of amino acids is extremely variable. Some organisms can decarboxylate them to produce free amines. Some of these are slightly toxic. A general belief has existed for many years that food poisoning may be caused by these amines or ptomaines. Even though it is well known that the ptomaines are not the cause of food poisoning, it seems very difficult to get the general public to accept this fact. Many still believe that ptomaines are the most common cause of food poisoning. Although this is not true, their presence in food to any extent certainly is a sign of food spoilage.

---

[1] A. I. Kendall, *J. Internal Diseases*, **30**, 211 (1922).

[2] Arthur T. Henrici, *Molds, Yeasts, and Actinomycetes*. Wiley, New York, 1930.

In attacking an amino acid, microorganisms may split off the amino group and liberate organic acid, alcohol, or hydrocarbon. Some of these can then be fermented to yield still other products including simpler acids, alcohols, and gases such as carbon dioxide and hydrogen. The liberation of ammonia from amino acids and their products is spoken of as ammonification. This may become extensive in advanced proteolytic decomposition.

The decomposition of proteins is usually associated with the liberation of odoriferous compounds. Sulfur is liberated as hydrogen sulfide or as mercaptans. Other odoriferous compounds such as indole, skatole, putrescine, and cadaverine are also frequently liberated. As a general rule, microbial spoilage involving the decomposition of proteins produces changes that leave little doubt that something undesirable has happened.

3. Fats

Fats are less susceptible to microbial decomposition than are carbohydrates and proteins. Extensive changes can, however, be brought about in fats.[3] Many microorganisms contain lipolytic enzymes that hydrolyze the fats to produce free fatty acids and glycerol, which then may be fermented in the same manner as carbohydrates. The molds as a class are more adept in this ability than are the bacteria, and in most foods where molds have been growing, extensive changes in the fats may be expected.[4] Thus we find that all cheeses ripened by molds have marked changes produced in their fats. Here again, as in carbohydrates, the breakdown of the fats by the microorganisms may or may not be regarded as spoilage, depending on the food habits of the people.

Fats may also undergo rancidification because of microbial action, although this latter change is more often caused by catalytic oxidation. This change involves a breakdown of the fatty acid portion of the fat with the formation of shorter-chained acids, aldehydes, ketones, and peroxides. Concurrently there occur changes in odor and taste that make the food undesirable.

---

[3] L. B. Jensen, *Microbiology of Meats*. Garrard, Champaign, 1942.

[4] Arthur T. Henrici, *Molds, Yeasts, and Actinomycetes*. Wiley, New York, 1930.

The classes of compounds that are products of microbial decomposition of carbohydrates, fats, and proteins are as follows:

From Carbohydrates: aliphatic acids, alcohols, aldehydes, ketones, carbon dioxide, hydrogen, methane.

From Fats: fatty acids, glycerol, aliphatic acids, aldehydes, ketones, alcohols, carbon dioxide, hydrogen, methane.

From Proteins: polypeptides, proteases, peptones, amino acids, amines, aliphatic acids, aromatic acids, aldehydes, ketones, heterocyclic compounds containing indole, phenols, mercaptans, hydrogen sulfide, ammonia, hydrogen, carbon dioxide, methane

4. Changes Involving Synthesis

In addition to the changes mentioned above, microorganisms may effect other changes that contribute to food spoilage. They may synthesize compounds that alter the flavor, odor, and color of the product to an undesirable degree. Thus, we find that many flavor changes in dairy products are not caused by the accumulation of products of decomposition, but rather by compounds elaborated by bacteria. The growth of *Pseudomonas pyocyaneus* in milk may change its flavor because of the formation of pyocyanine. The pigments elaborated by organisms can simulate all the colors of the spectrum.[5] Some of these have interesting historical backgrounds. The red color produced by *Serratia marcescens* has been blamed for some of the early wars. This organism growing on bread will produce a blood-red pigment. Milk may develop yellow, blue, or brown colors because of bacterial growth, and cheese can develop all kinds of colors, depending on the species of mold. Here again the color change may not always be regarded as a sign of spoilage.

Bacteria can also synthesize toxic products and thus spoil food. Botulismus toxin from *Clostridium botulinum* and the enterotoxin from the staphylococcus are examples of these. These will be discussed subsequently.

5. Changes Caused by Enzymes

The spoilage of food from enzyme action may be as varied as that caused by microorganisms; in fact, all microbial food spoilage is caused by the enzymes contained in or elaborated by these organisms. In speaking of enzymatic changes as distinct from microbial changes, it is customary to limit the enzymes to those that are normally present in the product and to ignore those formed by the contaminating organisms. Many food products contain diastatic enzymes which, when conditions are right, can change starches to sugar. Thus, if stored at too high temperatures, potatoes became sweet and soft. This could be looked upon as spoilage.

Most living cells contain autolytic enzymes that can act on proteins. Thus, when animal or plant tissue is stored, these enzymes can bring about extensive changes. The conditions that favor these enzymatic changes are also conditions that favor bacterial growth, so it is difficult to know which is the more important. Autolysis can be, and sometimes is, sufficiently extensive to be regarded as food spoilage. The casein in sterile unheated milk can be autolyzed by naturally occurring proteolytic enzymes.[6] Such milk would certainly be considered spoiled.

---

[5] B. W. Hammer, *Dairy Bacteriology*. Wiley, New York, 1928.

Recent studies on dehydrated foods have made it apparent that naturally occurring enzymes can spoil food. In these products, moisture is removed to a point where microorganisms can no longer grow. In spite of this, the product may undergo marked changes in flavor. This can often be avoided by blanching, which consists of preheating the product before it is dried to the point where the enzymes are inactivated Heating to 165° C. for a few minutes usually suffices. The blanched dried product keeps well, whereas the unblanched spoils, clearly showing the effect of the enzymes. It is not known just what enzymes are involved. Some of them can act on fats, but others are undoubtedly proteolytic. See Volume II, Chapter IX.

Many of these enzymatic changes set in as soon as the plant is picked or immediately after the animal is killed. The extent of enzymatic activity that must take place before the product can be considered spoiled varies with the product. Thus, green peas may spoil in a few hours even though they are placed in a cool room, while a beef carcass may be deliberately left in a similar place for many days to allow considerable autolysis to take place in order to make the meat more tender and thus more desirable.

Just as plant and animal tissues contain proteolytic enzymes, they also contain lipase. If the food is stored without first inactivating these enzymes, the fats will be considerably altered. The free fatty acids will increase progressively on storage. Here again, depending on the product, the change may or may not be classified as spoilage. Thus we will tolerate a considerable amount of free fatty acid in summer sausage or in cheese, while a similar amount in dehydrated meat would be considered a sign of spoilage. Likewise, in frying fats we would not tolerate much free fatty acid.

6. Oxidative Changes

Oxidative changes that are associated with food spoilage are probably nearly always concerned with the oxidation of fats. Other oxidative changes, however, are sometimes involved. Thus, the oxidized flavor of milk, according to Olson and Brown,[7] is initiated by an oxidation of ascorbic acid. In dehydrated vegetables and meats, oxidative changes, with or without enzymes, may bring about undesirable changes in flavor and color. Some of these are certainly not associated with the oxidation of fats alone.

The rancidification of fats has been considered for a long time as being caused by direct oxidation by atmospheric oxygen. Food in which the fats are rancid would be considered spoiled, and rightly so, by most people. Not only is the flavor unpleasant, but the rancid fats counteract or destroy certain vitamins in the foods. In addition, some of the by-products of oxidized fat may be toxic.[8] The products of oxidative rancidification are short-chained fatty acids, aldehydes, ketones, and peroxides.

---

[6] B. W. Hammer, *Dairy Bacteriology*. Wiley, New York, 1928.

[7] F. C. Olson and W. C. Brown, *J. Dairy Sci.*, **25**, 1027 (1942).

[8] G. O. Burr and R. H. Barnes, *Physiol. Revs.*, **23**, 256 (1943).

III. CONDITIONS AFFECTING SPOILAGE

The environmental conditions that affect food spoilage are varied and interrelated. Even though these must be discussed separately, they should be thought of collectively. The factors that must be considered are temperature, time of storage, initial contamination, chemical environment, gaseous environment, and moisture content.

1. Temperature

Temperature is perhaps the most important factor. Its effect on the growth of microorganisms is not unlike its effect on an ordinary chemical reaction, the rate of which is approximately doubled for each 10° rise in temperature. Within certain limits the same rule may be applied to bacteria, except that in the case of microorganisms the relationships are more complex.[9]

Several different groups of organisms are recognized, based upon their temperature requirements. Thermophilic organisms are those that grow best at temperatures above 40° C. or 104° F. Many of these will grow very well at temperatures up to 65° C. Mesophilic bacteria grow best at temperatures below 40° C., and generally not at all at temperatures much above that. Cryophilic organisms grow best at low temperatures. Some of these are inhibited even at ordinary room temperatures. All of these types may be concerned in food spoilage.

For each of these groups the bacteriologist recognizes certain cardinal temperatures. The temperature at which the organism grows best is called the optimum temperature. Above and below this, there are points at which the organism ceases to grow—the thermal inhibition points. If the temperature rises above the high thermal inhibition point, a temperature is soon reached which will kill the organism—the thermal death point. Most bacteriologists define the thermal death point as the minimum temperature that will kill the organism after an exposure of 10 min. Theoretically there should be a low thermal death point. It is very questionable, however, whether bacteria can be killed by freezing. Certainly, if they can, the point is too low to be of practical value. Ordinary freezing temperatures will not do this.

Although these cardinal temperatures may be easily defined, careful analysis will show that the concepts are not as simple as they may at first appear. For any one organism there are no fixed points; the values will vary with the environment and the method of determination.

In speaking of the thermal destruction of bacteria the term thermal death point is really meaningless. We should, rather, speak of the thermal death time. This emphasizes, as it should, both time and temperature—the higher the temperature the shorter will be the time required for killing. Also, the thermal death time will vary with the past history of the organism. Organisms that have been subjected to desiccation are more difficult to kill than similar organisms that have been suspended in water.[10] Closely allied with this, we find that bacteria can be destroyed at lower temperatures with moist heat than with dry heat. As an example, the food-poisoning staphylococcus will be killed at 70° C. in 10 min. when suspended in water, while it will resist 120° C. for the same length of time when desiccated and subjected to dry heat. Similar phenomena can be observed with most organisms. Other factors affecting the thermal death time are discussed in Volume II, Chapter XI.

---

[9] R. E. Buchanan and E. I. Fulmer, *Physiology and Biochemistry of Bacteria*. Vol. 2, Williams and Wilkins, Baltimore, 1930.

[10] A. T. Cameron, *Trans. Roy. Soc. Can.*, [3], Sec. V, 29, 53 (1930).

Likewise, the optimum temperature for growth is dependent upon the environment and upon the method of determination. For one thing, the values will be influenced by the composition of the medium. It has been shown, for example, that in the case of yeasts the optimum temperature will vary with the ammonium ion concentration.[11] The optimum temperature of growth of an organism may thus be different in one food product than in another. The optimum temperature that is recorded for an organism is also dependent upon how it is determined. If based upon the measurement of the population after several days of growth, the value will be different from a value based on a measurement after a few hours of growth. In general, the longer the interval of observation, the lower will be the recorded optimum temperature. For a detailed discussion of these variables, the reader is referred to standard texts on bacteriology.

Another temperature effect which deserves mention is the range through which microorganisms can grow. This varies considerably with different species of bacteria. Some of the mesophilic organisms, for example, that have an optimum temperature of about 30° C. may grow at a temperature as low as zero, and at temperatures as high as 40 or 45° C. Furthermore, these organisms may survive exposure to temperatures up to 120° C. Thus, these organisms can survive or multiply through a very wide range of temperature. On the other hand, some of the parasitic organisms are much more sensitive. Their optimum temperature may be approximately 35° C., but they will not grow at much below 30° C., and will be inhibited at a temperature of 45° C. They may be killed by temperatures not any higher than 65° C. These organisms, then, can exist only within a rather narrow range. In contrast to the mesophilic organisms, we find that the thermophilic type are unable to grow at ordinary room temperature or even at a temperature as high as 35° C. Many of these are spore formers and may require a very high temperature for thermal destruction.

2. Storage

From the above considerations, it is apparent that the type of organism which may grow in or upon food will depend upon the temperature at which the food is stored. If it is kept at a high temperature, growth may be limited to the thermophilic organisms. If it is stored at low temperatures, the thermophilic organisms will not be able to multiply, but instead some of the cryophilic organisms may grow to such an extent that the food is spoiled. Since these different organisms have entirely different effects upon the food, the changes produced will vary, depending upon the temperature at which the food is kept. For example, if milk is stored at ordinary room temperature, it will undergo normal souring and will not develop any off-flavors or odors other than those associated with the acids. If it is stored at low temperatures, cryophilic proteolytic organisms may develop that will attack the proteins rather than the carbohydrates.[12] As a result, the milk will not become sour but will develop bad tastes and odors. Another example deserves mention. In the canning of vegetables, the heat treatment may be sufficient to destroy all but the thermophilic spore formers. If the canned goods are kept warm for a considerable period of time the thermophiles may develop and cause spoilage, either a gaseous fermentation which will bulge the cans, or a flat sour. If the canned goods are quickly cooled to room temperature or lower, the thermophiles cannot develop and the canned product may then keep almost indefinitely. It is important for the food technician to bear in mind the different properties of these microorganisms, as such information may prove very useful in determining the proper handling of food to eliminate spoilage.

---

[11] R. E. Buchanan and E. I. Fulmer, *Physiology and Biochemistry of Bacteria.* Vol. 2, Williams and Wilkins, Baltimore, 1930.

The temperature of storage will have a great effect upon the rate of growth of the microbial flora. As mentioned earlier, the rate of growth is influenced by temperature in about the same way as ordinary chemical reactions. This is particularly true if the storage temperature falls within the growth range of the organisms. Within this range, the rate of growth will approximately double for every 10° rise in temperature. Thus, the rate of growth at 44° C. would be 16 times faster than at 4° C. The temperature coefficient of rate of growth is not constant; as a result, the actual difference in rate of growth at 4° C. and at 44° C. will be even greater. If we compare the rate of growth of cryophilic bacteria with that of the thermophilic, we find that the latter grow much more rapidly at their optimum temperature than the former. Since bacterial growth is only a composite of a large number of chemical reactions, this is understandable. In general, it may be stated that the higher the optimum temperature the more rapid the growth will be at that temperature. Since thermophilic organisms have such high optimum temperatures, it means that they grow very rapidly, and it is for this reason that one has to be careful to prevent food from being stored at temperatures that will allow these organisms to grow. This applies not only to the thermophilic organisms, but also to the mesophilic bacteria. Any food stored at moderately high temperatures may spoil in a few hours. Thus, it is important in the handling of all food to keep it at as low a temperature as practicable to avoid undue growth of contaminating organisms. Many examples of this can be cited. Milk that has not been properly refrigerated and stored at low temperatures will invariably be sour by the time it reaches the consumer. Packing plants have experienced time and again that if hog carcasses are not chilled promptly after slaughter, there will be a high percentage of ham souring during the cure. It has been found that this is caused by the excessive growth of microorganisms during slow chilling.[13]

---

[12] B. W. Hammer, *Dairy Bacteriology*. Wiley, New York, 1928.

[13] L. B. Jensen, *Microbiology of Meats*. Garrard, Champaign, 1942.

3. Initial Contamination

One of the important variables contributing to microbial spoilage of food is the amount of contamination to which the food has been subjected. To appreciate this, it is necessary to understand the rate of growth of bacteria. This can be illustrated by a curve in which the population is plotted on the vertical axis and the elapsed time on the horizontal axis (Fig. 26). The general shape of this curve will be the same irrespective of the organism involved or the composition of the medium. It may differ however, quantitatively, depending upon a number of factors. For the present we shall confine the discussion to the initial portion of the curve, representing a time when there is little if any increase in the population. This is called the lag phase. The length of this phase depends upon the size of the initial population, the temperature of incubation, and the environmental factors.[14] It is also influenced by the past history of the organism. If the initial population is low, the lag phase will be long. Thus we can see the importance of avoiding excessive contamination. Food that contains few organisms will keep a disproportionately longer time than food which is heavily contaminated. As a general rule, the lag phase will be the shortest at the optimum temperature for growth of the organisms, and will lengthen as we deviate from that on either side. The lag phase will be lengthened if anything is done to the environmental factors that will tend to make the medium a less favorable one for the growth of the organisms. It is perhaps for this reason that food which is not heavily contaminated may be preserved by chemicals that are not ordinarily considered toxic to microorganisms. The presence of these chemicals may so affect the lag phase that the keeping time is prolonged considerably. If the organisms present in the initial popu-

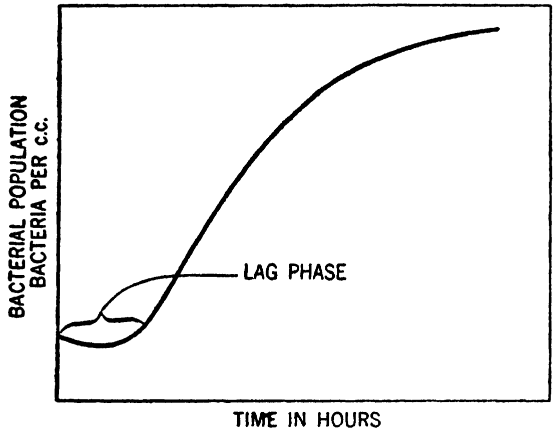


Fig. 26.—Initial phases of bacterial growth curve.

---

[14] A. T. Henrici, *The Biology of Bacteria.* 2nd ed., D. C. Heath, New York, 1939.

lation are organisms that have been recently obtained from an actively growing culture, the lag phase will be shorter than if they came from a medium in which they had been dormant. This has an important bearing on sanitary measures taken in a food establishment. To make this clear, let me cite some examples.

Let us suppose that meat is being processed on wooden blocks. This wood gradually becomes saturated with meat juices. At the end of the day's operations, the wood may be thoroughly cleaned, but a small amount of water may be left on the surface. The meat juices extracted from the wood by this water will furnish a good medium for the growth of bacteria, and during the night the organisms will grow luxuriantly in this medium. The next morning, when the block is again put into use, the first meat that comes along will be heavily contaminated by an actively growing culture. This meat will spoil quickly in spite of the sanitary precautions the operator believes he has exercised. To cite another example, a dairy may not completely clean out all of the pipe lines in its equipment. Water containing a small amount of milk may be left standing in some parts of the equipment. During the night, organisms find this a suitable medium for growth, and when fresh milk is put through the next morning, it will be heavily contaminated with an actively growing culture. This milk will then spoil quickly.

If food is so heavily contaminated as to produce a short lag phase, it may be virtually impossible to prevent that food from spoilng. The ordinary preservatives will probably prove inadequate, and because of the heavy population sufficient growth of microorganisms may take place even at low temperatures to bring about spoilage. It is apparent from the above considerations that proper preservation of food makes it necessary that strict sanitation be invoked to make possible a long lag phase for the growth of organisms.

Perhaps equally as important as the extent of contamination is the kind of contamination. It is frequently necessary to invoke rigid sanitation so as to prevent contamination by specific organisms. For example, dairy equipment may become contaminated by organisms which produce ropy milk. To avoid a specific type of food spoilage will necessitate a complete elimination of such organisms, and will require unusual sanitary precautions. In the canning industry, great care is exercised to avoid the contamination of the product by thermophilic bacteria. Not only is it necessary to eliminate such organisms as far as possible from the equipment, but it is also important that the raw materials contain few, if any, such bacteria. Numerous other cases of a similar nature could be cited. Special precautions may have to be taken to avoid contamination by organisms that can cause food poisoning.

4. Chemical Environment

The effect of chemical environment is very marked. Such things as acidity, salt content, osmotic pressure, the presence or absence of nitrates or nitrites, and the presence of accessory growth factors and antibiotic substances, all have their influences upon microbial growth.

(a) *Acids*

The effect of acids has already been discussed to some extent. Most bacteria prefer to grow in a neutral or slightly alkaline medium; nevertheless many organisms are acid-tolerant and can develop in media of moderate acidity. Acid tolerance among organisms varies considerably. In general, we can state that bacteria can usually grow at *p*H values ranging from 4.5 to 10. Of course not all bacteria can grow in a *p*H range as wide as this. Saccharolytic organisms are as a rule more acid-tolerant than proteolytic organisms, many of which will not grow at *p*H values much below 5.5 This interference with growth by acids is not solely dependent upon the *p*H. Organic acids usually interfere at higher *p*H values than the inorganic acids. It is believed that the unionized acid molecules have an inhibitory effect, and thus the *p*H alone is not a reliable criterion of toxic action. Of the organic acids, citric, acetic, and propionic acids are more effective than lactic or pyruvic acid.

The limiting *p*H of growth with any particular acid is also influenced by the composition of the medium. It is therefore not safe to draw conclusions as to what an acid will do in one food product from observations made on a different kind of food. The presence of salts of the acid in question will have a marked influence. Salts of other acids and proteins will also have some effect. A general rule is that any influence that tends to lower the ionization of an organic acid without changing its concentration will make that acid more toxic, and vice versa.

Changing the *p*H or acid content of a food product will affect not only the growth of the organism; it will also affect its heat tolerance and, in general, its biological activity. It is a well-known fact that canned goods are more easily preserved by heat sterilization if acids are present than if the food is neutral or slightly alkaline. As mentioned above, proteolytic activity even by acid-tolerant organisms is decreased in the presence of acids.

Yeasts and molds are as a rule more acid-tolerant than bacteria. Many yeasts and molds will grow at a *p*H value as low as 1.5. This fact is made use of in bacteriology for the isolation of molds and yeasts from mixed cultures. It is because the molds are so acid-tolerant that it is difficult to preserve food from mold spoilage by acids alone.

(b) *Sodium Chloride*

It has long been known that microbial growth and food spoilage is counteracted, to some degree at least, by common salt. As in the case of acid tolerance, salt tolerance of organisms varies considerably. Gram-negative bacteria seem to be much more sensitive to sodium chloride than the Gram-positive. The former are inhibited by concentrations of sodium chloride of 8% or less, while the latter will grow sometimes in a saturated solution. There are some organisms that are halophilic in nature and will not grow at all unless the salt concentration is high. This is the case with the microbial flora of the sea.

It is not clearly understood what makes common salt inhibitory. It certainly cannot be entirely attributed to its effect on the osmotic pressure, for salts other than sodium chloride even in much higher concentration do not show the same effect upon growth. The action of sodium chloride may be related to its dehydrating ability. With our present state of knowledge it might perhaps be best that we be satisfied with the statement that it is a specific action of sodium chloride. In attempting to predict what the effect of sodium chloride will be in a certain food product, one should calculate the concentration of the salt in the water that is present. This may not always be reliable because some materials, such as proteins, may bind some of the chemical and remove it from the sphere of action. It was mentioned previously that the beneficial effect of chemicals may be a result of their ability to lengthen the lag phase. This may well be the action of sodium chloride, because, once a food is heavily contaminated, it is no longer possible to preserve or check the growth of bacteria by reasonable increases in the salt content. In low concentrations sodium chloride as well as many other salts will have a stimulating effect upon bacterial growth. Thus we find that in the preparation of bacterial media, from $^1/_2$ to 1% of salt is sometimes added.

(*c*) *Nitrates and Nitrites*

Nitrates and nitrites are also frequently incorporated into foods, particularly into cured meat. Among food technologists it is generally believed that these two chemicals have a retarding effect upon microbial growth.[15] It is well known that many bacteria contain enzymes that can activate these two chemicals so that they will act as oxidizing agents. Media that would otherwise be anaerobic may, by their presence, be kept in a state of high oxidation-reduction potential, and thus maintain in effect an aerobic condition. Since a great many organisms prefer to grow where the redox potential is low, nitrate and nitrite may interfere with their growth. It is perhaps for this reason that nitrate and nitrite are beneficial in controlling food spoilage caused by putrefactive anaerobes. Nitrate is effective in this way in concentrations of 0.2% or less, and nitrite in concentrations of 0.02% or less. Since nitrites are more reactive and are used in smaller amounts, their effect will be only temporary if used alone.

(*d*) *Sugar*

It was mentioned in an earlier section of this discussion that sugar has a marked effect upon the type of changes that microorganisms produce in foods. In addition to the effects mentioned there, sugar may be used in such high concentrations that the organisms are hindered from growing by osmotic effects. High concentrations of sugar are needed for this purpose, and it is therefore impractical except in foods that contain a relatively small amount of water. To inhibit microbial growth, the concentration must be 65% or more in the water that is present. Even at this concentration, some organisms can grow, such as certain strains of yeasts, molds, and some gum-producing bacteria. Sugar is used in this way for the preservation of jams and jellies and sweetened condensed milk.

---

[15] L. B. Jensen, *Microbiology of Meats.* Garrard, Champaign, 1942.

5. Accessory Growth Factors

Our knowledge concerning accessory growth factors of bacteria and naturally occurring antibiotic substances is very limited. Nevertheless, these may be of considerable importance in connection with food spoilage and food preservation. Many people look upon the accessory growth factors as bacterial vitamins. In fact, many of the components of the vitamin B complex have been found to serve as accessory food factors for bacteria. It has been the experience of bacteriologists that these unknown substances are universally present in plant and animal tissue extracts in sufficient amounts to satisfy bacterial requirements. Therefore, if some foods are unfavorable media for the growth of bacteria, it is not likely that the reason is a lack of these accessory growth factors.

Inasmuch as bacteriologists are just beginning to learn something about the naturally occurring antibiotic substances, not much can be said about their influence upon food spoilage. It is an interesting topic for speculation, and the discovery of these substances has offered a plausible explanation of some phenomena that have been observed in food bacteriology.

It is a well-known fact that ordinary spoilage bacteria do not grow in living plant or animal tissue. In animal tissue the antibodies have been regarded as substances that interfere with bacterial growth. Antibodies are not found in plant tissue, so if there are chemicals there that prevent bacteria from growing, they must be of a different nature than the antibodies in animal tissue. Even in animal tissue we cannot account for the inability of spoilage bacteria to grow in living tissue. There are, of course, several possible explanations. The bacteria may not be able to compete with the animal or plant cells for the available food, nor may they be able to use the proteins as a source of nitrogen until they have undergone at least partial autolysis, which is prevented in the living cell.[16] It is certainly within the realm of possibility that there may be as yet unknown antibiotic substances in both plants and animals that interfere with growth of bacteria.

In recent years a large number of naturally occurring antibiotic substances have been discovered.[17] They seem to be widely distributed in nature, and have been isolated from bacteria, yeasts, molds, animal, and plant tissue. Some of them are able to inhibit growth of bacteria in very small concentrations, as, for example, gramicidin and penicillin, of more recent discovery, and pyocyanine, that has been known for some time. From studies that have been made in our laboratories upon the inhibiting effect of salt, it appears that salt is much more effective in animal tissue than one would expect. We have held the opinion that the salt may be acting synergistically with naturally occurring antibiotic substances.

---

[16] Marjory Stephenson, *Bacterial Metabolism.* Longmans, Green, London, 1939.

[17] R. J. Dubos, *Ann. Rev. Biochem.*, **11**, 668 (1942).

Spices have been used for many generations as an aid in food preservation. Some of these spices are known to be antiseptic, as, for example, the oil of thyme and oil of coriander. The antiseptic action of the essential oils present may be augmented by the antibiotic effect of as yet unknown compounds in the spices.

It has been shown that there are substances in cabbage, onions, and turnips that are toxic to *Escherichia coli* and *Bacillus subtilis*. These substances may also be toxic to other organisms.[17] The production of such antibiotic substances may be nature's way of controlling microbial growth. Perhaps a new door has been opened for research in food preservation.

6. Gaseous Environment

It has been mentioned that the presence or absence of air may affect the course of food spoilage. Obligate aerobic organisms will grow only in the presence of air, while anaerobic bacteria grow only in its absence. The vast majority of bacteria, however, grow almost equally well under either condition, although their mode of nutrition is different under the two conditions.

(*a*) *Oxygen*

In the presence of air, oxygen is the oxidizing agent that supplies energy for growth. Under anaerobic conditions the organic matter present must supply both the oxidizing and the reducing agent necessary for the release of energy. There may be certain conditions, therefore, under which organisms will grow only in the presence of air. Numerous cases have been cited in the literature in which a medium will support aerobic but not anaerobic growth of facultative anaerobes.[18] An experience with canned goods appropriately illustrates this point. The majority of commercially canned vegetables will keep in an incubator almost indefinitely. It would appear that they are sterile. If pin-point holes are punched in such cans and sterile air is allowed to enter, many of the cans will show spoilage upon subsequent incubation. After bacterial growth is evident, a variety of organisms can be isolated from the cans—aerobic, anaerobic, and facultative. A logical explanation is that the anaerobes and the facultative anaerobes do not grow in the sealed can because there are no suitable compounds present to satisfy their nitrogen or energy requirements. It is believed that the organisms are not able by themselves to attack the native proteins and break them down to suitable intermediates that can satisfy the organism. The enzymes that are responsible for the initial hydrolysis of the protein are exoenzymes, and these cannot be elaborated until the organisms begin to grow. When air is admitted, the energy.requirements of the facultative organisms are simplified and growth is initiated. Once growth has started, the necessary enzymes are elaborated for the breakdown of the proteins and then both the facultative and the anaerobic organisms find the material suitable for their use.

---

[18] B. C. T. G. Knight, *Bacterial Nutrition*. Special Reports, Medical Research Council, London, 1936.

Many nontechnical men in the canning industry perhaps have placed undue emphasis on the importance of oxygen removal from the can, believing it to be as important as the heat processing, if not more so. Bacteriologists, on the other hand, have had the tendency to regard the removal of air as of practically no importance. A compromise view is more nearly correct. In some exceptional cases in food preservation, the removal of air may in itself be sufficient. This would be true in the case of foods that can be spoiled by strict aerobes only, such as cider or alcoholic beverages. Acetobacteria can oxidize ethyl alcohol to acetic acid, and they are able to grow when the alcoholic content is high enough to prevent the growth of other types. These bacteria, however, can grow only in the presence of air, so the exclusion of air in this case will preserve the product. Molds as a class are aerobic, and many foods that will keep in the absence of air will spoil if exposed so as to allow mold growth. This was mentioned on page 358 in connection with sour milk and sauerkraut.

(*b*) *Carbon Dioxide*

The only gas other than oxygen that has any marked influence on the growth of bacteria in foods is carbon dioxide. Many varied and unfounded claims have been made for this gas—that relatively high concentrations of it can check microbial growth and thus preserve food. In particular, it has been claimed that carbonated milk will keep for long periods. Attempts have been made to preserve meat and other foods under an atmosphere of carbon dioxide.[19] In producing an atmosphere of carbon dioxide, the oxygen may be removed, which will affect the growth of obligate aerobes. Under relatively high pressures of carbon dioxide, the *p*H may be sufficiently altered to inhibit bacterial growth. There is no evidence from well-controlled experiments that carbon dioxide exerts any toxic effect except as it may change the *p*H or remove the oxygen. It is not likely, therefore, that carbon dioxide can act as a general inhibitor. On the contrary, it has been shown that many organisms will not grow unless some of this gas is present, as, for example, the brucella. From the studies of Werkman and Wood,[20] it is apparent that carbon dioxide can be assimilated by many organisms and may play a vital role in their nutrition.

7. Moisture Content

One of the very important conditions that influence bacterial growth is the moisture content. Bacteria must get their nutrients in a soluble form, and this means that water must be present. It is well known that dry or semidry foods do not spoil by bacterial growth. The limiting moisture content for bacterial growth is not a fixed value. It depends, like most other factors, upon the *p*H, salt content, and other environmental conditions. In the case of cereals, a moisture content of 15% or less does not allow bacterial spoilage. In dried meats growth is inhibited at a moisture content of from 18 to 20%, although in soil this moisture content will allow extensive growth. The difference must be due to water-soluble components that are present. The difference is even more striking if we compare soil to peptones. Bacteria will show very little growth in a peptone solution containing 45% water. The amino acids or other soluble constituents may be toxic in high concentrations, or there may be present some natural antibiotic substances that reach an effective concentration when the water content is low.

---

[19] T. Moran, F. C. Smith, and R. G. Tomkins, *J. Soc. Chem. Ind. London*, 51, 164T (1932).

[20] C. H. Werkman and H. G. Wood, *Advances in Enzymology*. Vol. 2, Interscience, New York, 1942, p. 135.

When salts are present, the limiting moisture content is higher. Thus summer sausage keeps very well even at moderately high temperatures with a moisture content of from 35 to 40%. In recent studies in our laboratories we placed summer sausage in hermetically sealed cans and stored them at room temperature for varying periods of time up to a year and a half. At periodic intervals some of the cans were opened and bacterial counts were made on the meat. It was found that when 3.5% salt was added to the original meat, which was then dried to a moisture content of 35%, the bacterial population decreased continuously during storage. In the absence of salt the moisture content had to be lowered to less than 24% to get a similar drop in population.

The effect of moisture on spoilage is important in connection with the preservation of dehydrated foods. It has been found that, as far as bacteria are concerned, the moisture content need not be below 10 to 15%. However, with moisture content this high, enzymes or oxidative changes may bring about spoilage. Thus, in many of these products, bacterial spoilage may not be of primary importance.

Molds are able to grow on products that contain less moisture than is required by bacteria, providing there is sufficient moisture in the air surrounding the food. With the relative humidity fairly high, the molds will develop on products that contain very little water. Summer sausage that is well preserved against bacterial spoilage will allow molds to grow when the humidity in the air is 60% or higher. A control of the humidity is an important means of controlling mold growth on such semidry products. The rate of air velocity will have some influence on the limiting humidity. With stagnant air, a layer of moisture-laden air can surround the product so that the humidity is really higher where the molds are growing than where the measuring instrument is hung. The same effect is produced if the product is placed in a container without ventilation. The humidity in the container will become very high and will allow molds to grow whereas, under proper ventilation, this might not occur.

IV. ENZYME SPOILAGE

Enzymes are influenced by their environment in about the same way as bacteria. They have an optimum temperature and a thermal inactivation point. As in the case of microorganisms, enzymes differ as to their cardinal temperature. Like the bacteria, the enzymes are influenced by the *p*H and other factors in the chemical environment. Each may have its own optimum *p*H and *p*H range through which it can act. Salts may retard or enhance their activities, depending upon the concentration and the kind of enzyme involved. Many chemical inhibitors of enzymes are known, but up to the present, these have not been employed to prevent enzyme action in foods. Such use might be justified in a few instances. Before they could be used in foods, however, it would be necessary to prove that they were noninjurious.

In the case of dehydrated foods, the action of enzymes may be of primary importance. Here the moisture content is low enough to prevent bacterial growth, but the enzymes still act. It would, of course, be possible to remove enough water to prevent even enzyme action, but this might prove expensive and undesirable from the standpoint of rehydration. The only practical method of dealing with these enzymes now is to blanch the products before they are dried. The ideal is to heat the product just enough to destroy the enzymes, but not enough to harm it. Most food technologists have assumed that 165–170° F. for 10 min. will suffice. Such heating does destroy many enzymes but it may not destroy all of them. It has been shown by some investigators that some of the exoenzymes acting on proteins are fairly heat-stable and may resist even 212° F.[21] for short periods. Furthermore we know that many bacteria will not be destroyed by the blanching process commonly used. These organisms contain enzymes of various types and those we know are not destroyed. If they were, the organisms would be incapable of further growth. Possibly we need more experience with dehydrated foods before we can be certain that the present blanching techniques are adequate. Since the hydrolytic enzymes are the most heat-resistant, it is possible that they will be more readily affected by dehydration, in which case they would prove unimportant even if left intact.

V. OXIDATIVE CHANGES

Oxidative changes have already been mentioned briefly. These changes will also be influenced by the environment in much the same way as microorganisms and enzymes. The role of oxidation will be markedly increased at elevated temperatures. Here we are dealing with a straightforward chemical reaction, so there will be no thermal inhibition point. The rate of oxidation will increase with increases in temperature. The chemical environment may have a marked influence. Some salts may activate the oxidation, while others may retard it. Sodium chloride and nitrates both exhibit an enhancing action. Peroxides are oxidation catalysts and, if present in small amounts, will hasten oxidation considerably. As soon as oxidation sets in, more of these peroxides will be formed and thus these substances are autocatalytic.

Negative catalysts or antioxidants have the opposite effect. Small amounts of effective antioxidants may prevent oxidation for long periods of time. These substances may be synergistically affected by other compounds which in themselves are not antioxidants.[22] The exact role of the antioxidants and their synergists is not known. The mechanism of action may differ with different compounds. They may use up the oxygen or they may break the chain in the series of reactions that take place during oxidation. In considering oxidative changes, one's first thought is to remedy the matter by excluding air from the product. This proves effective in many cases, but unless the product is handled away from air all the time, enough oxygen may be adsorbed to bring about marked changes even though attempts are made later to exclude the air by evacuation.

---

[21] R. E. Buchanan and E. I. Fulmer, *Physiology and Biochemistry of Bacteria.* Vol. 3, Williams and Wilkins, Baltimore, 1930.

[22] H. S. Olcott, *Oil & Soap*, **18**, 77 (1941).

The oxidative changes may be related to microbial activity. As a result of the growth of organisms, compounds may be formed that can be oxidized to produce bad tastes and odors. On the other hand, products of bacterial growth may adsorb the oxygen or react with it to prevent the oxidation of compounds that produce bad tastes. Oxidized flavors, for example, will develop more easily in milk in which there has been no microbial activity than in milk in which bacteria have been grown.[23]

VI. PREVENTION OF SPOILAGE

In general, spoilage of foods can be prevented by taking advantage of all those conditions that inhibit microbial growth without injuring the food. Not all conditions can be taken advantage of for each product. It is of great importance to use those that are best suited for the conditions under which the product is to be handled. The subject of preservation of food is discussed in Volume II, Chap. IX.

1. Refrigeration

One of the most effective methods of preventing spoilage is refrigeration. Indeed, for many foods this is the only effective means. Foods that cannot be frozen should be stored at temperatures ranging from 32 to 38° F. At this temperature the growth of bacteria will be slow. If it is not heavily contaminated the product should keep for several weeks at this temperature. The length of time it can be stored depends, however, on the type of food. Milk will not keep as long as meat. If the food can be frozen without injury, the keeping time can be prolonged considerably. Growth of bacteria can be practically eliminated if most of the water in the food is frozen.[24] Upon defrosting, however, the food will usually be a better medium for growth than it was before it was frozen, and may spoil faster than fresh food of the same type. Storage at low temperatures not only slows up bacterial growth, but has a similar effect upon enzymes and oxidative changes.

2. Sterilization

Next to refrigeration, sterilization is the most common method of preservation. The preservation of canned goods depends upon this method. It is important here to heat the product to a temperature high enough to kill all organisms that can grow in the hermetically sealed product. This must be done after the can has been closed.

---

[23] L. M. Thurston and H. C. Olson, West Va. Agr. Expt. Sta., *Bull.* 255 (1933).

[24] L. B. Jensen, *Microbiology of Meats*. Garrard, Champaign, 1942.

The length of time of heating and the temperature that must be used will vary with the product and the size and shape of the container. In nonacid products, the temperature should reach 240° F. inside the can and be maintained for several minutes. A lower temperature, however, with a longer period of exposure may give equivalent results. This heat treatment is necessary to ensure the destruction of *Clostridium botulinum* and other spore-forming spoilage organisms.[25] Even with such drastic treatment, the more heat-resistant thermophilic spore formers may survive. By avoiding excessive contamination from these latter and by cooling the cans rapidly after processing, spoilage from the thermophils can be largely avoided.

The length of time required for processing will vary with the size of the container and the consistency of the product. It is important to heat the can long enough to produce a killing temperature in all parts of the can. The larger the container and the more viscous the material, the slower will be the heat penetration, thus necessitating a longer time of heating. The only safe way to determine the correct processing time for any given type of can or product is to measure the inside temperatures by means of thermocouples. Once the correct time has been established, it must be remembered that this will hold only as long as the initial temperature of the product is controlled. A can of cold material will heat somewhat more slowly than one that is already warm.

Nonacid foods cannot be sterilized at a temperature of 212° F. within any reasonable length of time. The cold pack, or any other nonpressure-cooking method, frequently used in home canning, is not to be recommended. Nonacid foods processed in this way are not sterile. The percentage of spoiled cans is likely to be high and there is always the potential danger of botulism. If it is necessary to resort to such processes in the home, it is of paramount importance that the product be handled with rigid sanitation; that the product be cooked in cans under water for not less than 4 hrs.; and that the product be boiled for at least 15 min. after removal from the can and immediately before being used.

Acid foods having a *p*H of 5.5 or less and meats cured with salt and nitrate can be processed at lower temperatures. In the case of acid fruits, processing at the temperature of boiling water will suffice, and cured meats will keep if processed to an inside temperature of 220° F. for 10 min., or its equivalent. Such products will not be sterile, but the surviving organisms will be mostly in the spore form that will not germinate in the presence of acid or cure. This does not mean that the processor can be careless with such products. Improper handling may result in a reduction of the acidity, and curing must be rigidly controlled to ensure proper keeping of the product in the can.

3. Pasteurization

Some foods cannot be sterilized without altering their normal flavor and aroma. A good example is milk. Such products may be heated just enough to destroy the pathogenic bacteria that may be present. This is called pasteurization. The common heat treatment is 143–145° F. for 30 min., or 160° F. for 15 seconds. Such treatment does not materially improve the keeping quality of a product like milk, but it may prove effective in beverages which in themselves are unfavorable for microbial growth because of the presence of alcohol or acids.

---

[25] J. R. Esty and K. F. Meyer, *J. Infectious Diseases*, **31**, 650 (1922).

4. Use of Chemicals

The use of chemicals for the preservation of food is not regarded as good practice, and is generally prohibited by pure-food and drug laws. There are a few such practices, however, that have been handed down to us from antiquity and are still widely used. Salt, sugar, nitrate, and nitrite are used in the curing of meat. Organic acids are added in pickling, and are produced in fermented foods and in cheeses.

In the curing of meat, one cannot rely upon chemical ingredients alone. While the cure is being incorporated into the meat, spoilage must be prevented by refrigeration at from 32 to 45° F. One purpose of the nitrite is to fix the color in the hemoglobin. In many cases the nitrite is formed from the nitrate by microorganisms. This means that a selected flora must be developed in the curing pickle. If the temperature is too low, the desirable organisms may be retarded and proper color fixation does not take place. Too low a temperature also will retard the penetration of the curing ingredients. If the temperature is too high, spoilage organisms will grow before the food is adequately cured.

In order to preserve meat so that it will keep under adverse conditions, it is necessary to incorporate into the product a fairly high concentration of salt; it must be so high, in fact, that the meat is almost too salty to eat. A concentration of 4% is about the maximum for palatability; a concentration of 5% is too salty. To retard bacterial growth, the concentration should be at least 3%, preferably 3.5%. The workable range in cured meat is therefore very narrow. Even with a concentration of from 3 to 4%, the product will not keep under adverse conditions and therefore should be kept under refrigeration. With the older method of curing in which the meat was immersed in a saturated brine, it was difficult to control the final salt concentration, which would generally be more than 4%. In recent years it has become common practice to inject the curing mixture into the vascular system. By this method the salt concentration can be controlled so as to be suitable for the taste of the average individual. As a matter of fact, there is now the danger of using too little salt. The public still is under the misapprehension that cured meat will keep at elevated temperature (see page 453), though mild cures do not provide this protection. The number of cases of food poisoning from cured meats have therefore increased. Certainly the practice of leaving mildly cured hams in warming ovens in restaurants until they have been used up should be discontinued. Such practice is dangerous, since the salt concentration is not high enough to give protection under such conditions. In fact, it would be better if the public regarded the mildly cured meats as perishable as fresh meats.

The use of chemical preservatives is discussed in Volume II, Chapter XIII.

5. Dehydration

In other cured meats, such as summer sausage, reliance is placed not only on the curing ingredients but also on dehydration. As mentioned previously, this increases the salt content in the water that is left and thus makes the environment less favorable for the organisms. Here again it is important to control the temperature during curing and dehydration so that the product does not spoil before the cure is completed. It is also important to avoid excess contamination. Heavy initial contamination may make it virtually impossible to prevent the meat from spoiling during processing.

We are dependent upon preservation through dehydration to a greater extent than the average individual may think. All of our dry foods owe their keeping quality to this. Such foodstuffs as cereals, flour, sugar, and fats would all be susceptible to microbial spoilage if they contained more water. The effect of water content upon bacterial growth has already been discussed on page 372. This method of preservation may be extended to other foods when we have learned to produce dehydrated products that are acceptable to the public. Great advances have been made, but it remains to be seen whether these dehydrated products are of such quality as to receive public acceptance.

6. Hermetically Sealed Containers

Food spoilage resulting from oxidation can be avoided by keeping the foods away from air in hermetically sealed containers. Spoilage chiefly due to rancidification can thus be avoided. By this means it is possible to extend the keeping time of fats and foods such as coffee, dried meats, and bacon, which contain fats that can become rancid. Another method of avoiding such spoilage is to use fats that are protected by antioxidants, or to add antioxidants to the food itself. This has not been used much in the past, but it may find more extensive use in the future. Removal of the air will also aid in preventing mold growth on foods that are otherwise protected from bacterial spoilage. Thus the canning of semidry sausage might be advisable.

7. Sanitation

In all types of food preservation, sanitation is very important. To obtain and maintain bacteriological cleanliness involves much more than the mere removal of dirt. Equipment must not only be washed clean, but it must be left in such a condition that new populations of organisms cannot develop during the time it is not in use. If water, either in large or small quantity, is left in or on the equipment, bacteria may grow in such numbers as to contaminate very heavily the subsequently processed food. Equipment used in food industries should be designed with an eye toward sanitation. Irremovable sagging pipe lines, valve pockets, inaccessible equipment, and complex mechanisms that cannot be cleaned should all be avoided (see Volume II, Chapter V). Unfortunately, this aspect of design has been too often overlooked. Properly constructed equipment alone is not sufficient protection and intelligent supervision of sanitary procedures is always necessary. Liberal use of clean water, detergents, disinfectants, and steam should be encouraged and the equipment should be left dry during idle hours (Volume II, Chapter VI).

VII. ANALYSIS OF FOODS FOR SPOILAGE

1. Bacteriological Analyses

There are very few quantitative tests that can be used to determine whether or not food is spoiled. Bacteriological analyses have been used for this purpose, but the results obtained are not as definite and reliable as is desirable. A quantitative determination of the bacterial population is helpful. When food is spoiled because of bacterial growth, the population is nearly always high. In order to interpret these counts, it is necessary to know the bacterial population in similar food that is known to be good. High counts do not necessarily mean that the food has spoiled, but they do mean that the food has been improperly prepared or handled, and that the potential keeping time has been reduced. On these grounds alone one may be justified in rejecting such food for public consumption. There are very few cases in which we have enough data to allow the establishment of standards for normal foods. About the only ones for which there are such standards are the dairy products, and these are practically limited to milk and butter. In the case of milk the standard is based on the total bacterial count, and in butter on the yeast and mold count. Even here it is not possible to use the same standard for all parts of the country, because the age of the milk delivered to the consumer will necessarily vary with the size of the community.

In spite of the fact that there are no standards, a bacteriological analysis will be helpful to the food technologist. If such tests are made frequently, a worker will soon be able to make rough standards of his own. The problem is made more difficult by the fact that not the numbers of bacteria alone, but the kinds of bacteria as well, determine whether or not a product is spoiled. With mixed cultures such as one finds in most foods, it is very difficult to get quantitative data on any particular species. The number of coliform organisms, however, is frequently used as a criterion of wholesomeness. The total number of bacteria, as determined by the standard plating technique, and the number of special types as determined by the extinction dilution technique[26] provide useful information both for general analysis and for special problems. For details of these methods, the reader is referred to texts on bacteriology.

2. Organoleptic Tests

Organoleptic tests will probably always remain the most useful tests in determining the general condition of food. For the layman these tests are very unreliable, but in the hands of an experienced technician they can become very accurate. Many foods are graded on the basis of such tests, and certain kinds of spoilage as, for example, putrefactive changes in meat, can be detected in the very early stages. In fact, government inspectors in packing plants, who have to pass or reject meat, become very proficient with the organoleptic tests. This type of analysis must be learned by actual experience, under proficient guidance.

---

[26] H. O Halvorson and N. R. Ziegler, *Quantitative Bacteriology*. Burgess, Minneapolis 1938.

3. Chemical Analyses

Certain kinds of spoilage can be detected and analyzed for in the laboratory. Food-poisoning bacteria can frequently be isolated from food. This will be discussed further in connection with food poisoning.

When fats become rancid, peroxides are formed which can be detected and determined quantitatively. Together with the organoleptic tests, such analyses are very useful. If the fats have been hydrolyzed the amount of free fatty acid present can also be determined quantitatively. A determination of the *p*H is also very useful. In a fresh sample of a given food, the *p*H value found will be characteristic of that food. If a value is obtained that is markedly different, it is a good indication that undesirable changes have taken place. For example, a determination of the *p*H of milk will tell whether souring has set in before it is discernible by taste or appearance. The *p*H of canned goods may indicate whether flat souring has set in. For such analyses, any standard electrometer may be used that employs a glass electrode. Undesirable microbial changes may produce significant amounts of volatile acids or ammonia. A determination of these may prove helpful.

VIII. FOOD POISONING

Food poisoning has been of vital concern to man for untold generations. Cases of food poisoning are relatively numerous today, and probably have been even more common in the past. Although caused by a variety of agents, most of these cases have been labeled ptomaine poisoning ever since 1870 when Spelmi, the Italian toxicologist, first used the term. Ptomaine was supposed to be a toxic product of the bacterial decomposition of protein, and there was a tendency to associate food poisoning with putrefaction. It is now known that the concept of ptomaine poisoning is false, and it would probably be much better if the term had never been introduced. In spite of the fact that the causative agents of food poisoning are now well known, many people—among them physicians—still persist in the belief that ptomaines are frequently the cause of food poisoning. One of the best arguments against this belief is the fact that people do not become ill from eating limburger cheese, in which there are many putrefactive bacteria and in which the proteins have undergone extensive breakdown. Furthermore the very organisms that are supposed to be responsible for ptomaine poisoning are present in our intestinal tracts. These organisms are probably producing the same substances from proteins in our intestinal tract which they are capable of producing in food. If ptomaine poisoning were a reality, we would all be subjected to it very frequently.

The types of food poisoning can be classified as follows: (*a*) those resulting from chemical poisons, (*b*) those resulting from poisonous plants or animals, (*c*) those resulting from toxins elaborated by bacteria. In addition, certain intestinal infections are caused by bacteria that are transmitted through food.

1. Chemical Poisons

There are many chemicals which are poisonous to man when taken by mouth. Those most commonly encountered in food are arsenic, certain alkaloids such as strychnine, sodium fluoride, and salts of some of the heavy metals such as mercury, cadmium, and antimony. Antimony poisoning has resulted from eating foods prepared in cheap cooking utensils, and cadmium is sometimes introduced into the food from cadmium-plated cooking utensils. Sodium fluoride, commonly used as an insecticide, has been mistaken for baking soda or grated bread crumbs and placed in food.

2. Poisonous Plants and Animals

A number of plants and animals are poisonous to man.[27] Some cases of food poisoning have been traced to mussels obtained from the Pacific coast during certain seasons of the year. It is well known that many varieties of mushroom are poisonous, and these are frequently mistaken for edible types. Ergotism is a poisoning from a certain fungus that grows on wheat or rye. Favism is associated with eating a certain bean or inhaling pollen from the flowers of the mother plant. Snakeroot poisoning results from drinking milk from cows that have been pastured in areas where the snakeroot is abundant. Some plants produce oxalic acid in sufficient quantities to cause poisoning. It is believed that this is the cause of rhubarb poisoning which has occurred when people have tried to use rhubarb leaves for greens.

3. Bacteria

Most cases of food poisoning are caused by bacteria which produce a toxin in the food. This is the case in botulism and staphylococcic food poisoning. Some cases of so-called food poisoning, however, are in reality infections, as the infections caused by *Salmonella* from contaminated food.

(a) *Botulism*

Botulism is caused by food in which *Clostridium botulinum* has grown and in which the toxin is still present. The organism is a spore-forming, anaerobic, soil saprophyte. It is noninfectious and incapable of causing symptoms of poisoning in the absence of the toxin. When the organism is permitted to grow in food, a powerful toxin may be elaborated, and if the food is then consumed, botulism will result. Since the organism is a strict anaerobe, it will not grow in food unless the oxygen is effectively removed. Toxin formed by the organism is not very stable. It is decomposed by some enzymes of other bacteria, and is also destroyed by heat.[28] Botulism will therefore be contracted only if special conditions prevail. The environment must be anaerobic, and some of the competing organisms that would destroy the toxin must be eliminated. Such conditions may be found in improperly cured meats, but they are more likely to be encountered in canned goods that have been improperly processed.

---

[27] F. W. Tanner, *Food-Borne Infections and Intoxications.* Garrard, Champaign, 1933.

[28] G. M. Dack, *Food Poisoning.* Univ. of Chicago Press, Chicago, 1943.

Clostridium botulinum is a Gram-positive rod that forms a central or subcentral spore. Like many other Gram-positive spore formers, the Gram-staining quality may be lost in old cultures. The organism is usually classified with the putrefactive or proteolytic anaerobes, although it does possess considerable saccharolytic power. When it is using sugar as a source of energy, one of the common end products is butyric acid. The odor of butyric acid is therefore very frequently associated with foods that have caused botulism. Butyric acid is not always produced, however, and since the organism is not a very active proteolytic type, putrefactive odors may not be associated with the growth of this group. In many cases, the food responsible for the outbreak of botulism has appeared normal as far as odor and taste are concerned. *Clostridium botulinum,* however, should not be looked upon as a single species. There are several different types of the organism, each producing a toxin which is immunologically distinct. Five of these types have been recognized to date. They are designated as A, B, C, D, and E. A guinea pig immunized to one type will produce an antitoxin that will neutralize the toxin of that type only. There are some reports in the literature indicating that cross neutralizations of the toxins sometimes do occur. This may be because the investigator was dealing with a mixed culture, and really immunized his animals with two toxins when he thought he was using only one.

Most cases of botulism in man are caused by Types A and B. A few cases have been attributed to Type E. Type D is associated with the African cattle disease called lamziekte, and Type C is responsible for the western duck sickness. There have been no known cases of botulism in human beings caused by these last two types.

The organism is widespread in nature. It has been isolated from the soil in practically all parts of the world. Whether it is present in all soil at all times is hard to say. There have been numerous cases in which people have failed to isolate the organism from soil. With the very large mixture or organisms in soil, however, it is sometimes very difficult to isolate any specific species. The failure to isolate the organism from a given sample does not necessarily mean that the organism is not there. It is believed that Type A botulinus is found chiefly in mountainous regions and in virgin soil, whereas Type B is found more often in cultivated soil. Type C seems to be associated with alkaline soil, such as is found in the western part of the United States, chiefly in Montana and neighboring States.[29]

The spores of many of the strains of *Clostridium botulinum* are very resistant to heat, particularly if they have been subjected to desiccation. According to Esty and Meyer,[30] the maximum heat resistance of spores of *Clostridium botulinum* is 4 min. at 120° C., 32 min. at 110° C., and 330 min. at 100° C. This, however, should not be looked upon as a constant characteristic of the spores. Their thermal death time, like that of most bacteria, will depend upon the composition of the medium in which the heating occurs. The heat resistance is decreased in the presence of acids and in concentrations of sodium chloride of from 8 to 20%. In low concentrations of the salt, from 1/2 to 1%, the heat resistance may be increased. Like most spores after they have been subjected to heat, they may be very slow in germinating. Dickson and his coworkers reported one case in which the spores did not germinate until 330 days after they had been heated.[31]

---

[29] M. F. Gunderson, *Proc. Soc. Exptl. Biol. Med.,* **30,** 747 (1933).

[30] J R. Esty and K. F. Meyer, *J. Infectious Diseases,* **31,** 650 (1922).

The toxin of *Clostridium botulinum* is a typical exotoxin against which antitoxins can readily be formed. The symptoms produced by the toxins are similar, despite their immunological differences and despite the biochemical differences in the strains. They are primarily neurotoxins that have an effect similar to that of curare. Like so many other exotoxins of bacteria, the toxins of *Clostridium botulinum* are heat-sensitive, and are rendered nontoxic by heating to 80° C. for a few minutes. With long heating, they can be destroyed at temperatures as low as 65° C. This is one of the redeeming features of the botulinus toxin. If food were always adequately cooked, there would be no danger of this type of food poisoning.

Botulism was first recognized as a specific type of food poisoning in Europe. Here it was thought of as a special type of meat poisoning, the first case being traced to sausage. Since the poisoning was first recognized in Europe in 1735, outbreaks of botulism have occurred throughout the entire civilized world. In Germany most of the outbreaks were traced to sausage; in Russia to fish, either smoked and dried or preserved by salt. Most of the cases that have occurred in the United States and Canada have come from improperly processed canned goods. According to K. F. Meyer, there were 359 outbreaks recorded in the United States and Canada from 1899 to 1941, comprising 1024 cases and 669 deaths.[32] Most of the cases in this country, and in recent years all the cases, have resulted from canned vegetables, and the majority of these from home-canned string beans. That this is so is easy to understand. Commercial canners are well aware of the dangers of botulism, and use cooking temperatures that ensure the destruction of the organism. When the housewife cans vegetables in the home, and uses the cold-pack or other methods in which the temperature does not go above 212° F., it is very likely that the spores of the organism will survive. Were it not for the fact that most people heat their canned vegetables before they use them, the number of cases of poisoning from home-canned foods would be much greater than it is today.

A number of different symptoms may result from the ingestion of toxins of *Clostridium botulinum*. Before the onset of symptoms involving the central nervous system, there may be nausea and acute digestive disturbances and vomiting. This nausea usually occurs within 24 hrs. of eating the poisoned food. Diarrhea may also occur early. In the later stages of the disease, constipation is more common. The typical symptoms of botulism are those involving the central nervous system. Disturbances such as double vision, or diplopia, difficulty in swallowing, paralysis of the involuntary muscles, and respiratory failure are all common. Death is usually due to respiratory failure. In most fatal cases, the patients have clear mental reactions up to the time of death. Duration of the illness in fatal cases is from 3 to 6 days after ingestion of the poisonous food, and in the nonfatal cases recovery is involved and slow.

---

[31] E. C. Dickson, G. S. Burke, D. Beck, J. Johnson, and H. King, *J. Am. Med. Assoc.*, **79**, 1239 (1922).

[32] G. M. Dack, *Food Poisoning*. Univ. of Chicago Press, Chicago, 1943.

The only known specific therapeutic agent for botulism is the antitoxin. Its use should therefore be encouraged. The difficulty, however, is that the patient does not usually know that he has consumed toxic food until the symptoms appear, and then it may be too late to expect results from the use of antitoxin. If antitoxin is used, 50,000 units should be injected intramuscularly after the patient has been tested for hypersensitivity. In outbreaks involving several people, it is not likely that all the people will become ill at the same time. It is therefore well to administer antitoxin to all the people who had participated in the meal that made the patients ill. Unfortunately, there are no other reliable treatments for the intoxication. Alcohol has been suggested and used, but its value is very questionable.

The laboratory diagnosis of botulism is not always easy, as the suspected food may have been discarded. In analyzing food, one must not rely only upon odor and appearance of the food, although these criteria may be very useful as an adjunct to other laboratory tests. The presence of toxin is determined by injecting watery extracts of the food into mice. To obtain the extracts, the food should be ground with sand in a mortar and the suspension should be centrifuged at high speed for $1^1/_2$ to 2 hrs. The mice which are protected by the various types of antitoxin should be injected with $^1/_2$-ml. portions of the supernatant fluid intraperitoneally. Those which are protected by the proper antitoxins should survive, whereas all of the others should die with typical symptoms of botulinus poisoning. In addition to demonstration of the toxin in the food, the organism itself may be isolated, using the procedure outlined by Dack.[33] Isolation of the organism with a failure to demonstrate the toxin does not constitute proof that the food was the cause of botulism. Food may frequently be contaminated with spores of *Clostridium botulinum*, but where the organisms have not grown, there will be no toxin, and no poisoning is possible.

Botulinus food poisoning can be controlled by destroying the spores in any food that will provide a suitable medium for their growth and that is to be stored in such a way that the organisms can develop and produce toxin. The danger of botulinus poisoning is now well recognized among all commercial canners, and commercial canning practices are generally safe as far as this type of poisoning is concerned. But many of the home-canning methods are not safe in this respect. Certainly the cold-pack method of canning, or the other methods that do not employ the pressure cooker, should not be recommended for the canning of vegetables. Even where pressure cookers are used, the process may not always be safe, since the temperatures attained may not be high enough to destroy the spores. The cooker may not be properly exhausted, in which case the pressure recorded on the gage is not a reliable criterion of the temperature.[34] Many of the gages on these cookers are cheap and unreliable instruments. The cookers should really be provided with thermometers as well as pressure gages, and the thermometer should be installed in or near the vent of the cooker, which should not be closed until a temperature of nearly 212° F. is reached. Another important step in the control of botulism is to take advantage of the heat sensitivity of the botulinus toxin. All home-canned vegetables should be heated to a boiling temperature for at least 15 min. before the vegetables are used. If this were universally practiced, there would be few cases of botulism even though inadequate processing had been used.

---

[33] G. M. Dack, *Food Poisoning*. Univ. of Chicago Press, Chicago, 1943.

(*b*) *Staphylococcic Food Poisoning*

Staphylococcic food poisoning, like botulism, is produced by toxins elaborated in the food before it is ingested. It is probably the most common of all the various types of food poisoning. Many of the cases are not very severe, and are never called to the attention of physicians or health authorities. There are probably very few adults who have not at one time or another suffered from this type of food poisoning. The disease is milder than botulism; the symptoms are of short duration and the recovery is usually rapid and complete. Mortality is extremely low. Most cases have probably been erroneously reported as ptomaine poisoning. About the only cases that come to the attention of health authorities are outbreaks involving large groups.

The organism responsible for this type of food poisoning is very widely distributed. It is found on the skin and in the nose and throat of most individuals. It is the common cause of boils, pimples, and carbuncles, and may be responsible for some forms of generalized infection. Not all staphylococci are capable of producing the enterotoxin that is responsible for the poisoning. Many strains have been reputedly tested for enterotoxin on human volunteers, and have never been found to produce it. Much of this work has been done by Dack and his coworkers.[35] The percentage of strains of staphylococci that cannot produce this toxin is not known, because to date there is no truly satisfactory test for differentiating toxin producers from the nontoxin-producing strains. It was believed for a time that the two types could be differentiated from each other by the hemolytic activity of the toxin producers and by their ability to liquefy gelatin. Work in recent years has shown that these tests are not reliable. Jordan[36] has concluded from careful studies that there is no way of differentiating the toxin producers from the nontoxin producers by biochemical tests.

Staphylococci are spherical organisms that usually grow in clusters like grapes but may frequently be found as diplococci or even in short chains. They are Gram-positive and produce no spores. They are more resistant to heat and more salt-tolerant than most nonspore-forming organisms, and are fairly acid-tolerant. As mentioned before, they are very widely distributed in nature and can be isolated from most environments and from most foods. Some strains will survive exposure to 70° C. for 10 min. Here, as in the case of other bacteria, the heat resistance will vary with the composition of the medium and with the past history of the organisms. The enterotoxin produced by staphylococci is somewhat unique when compared with the exotoxins of other bacteria. It is questionable whether this toxin is antigenic, as it is difficult to demonstrate the production of antitoxin. Unlike most exotoxins, the enterotoxin of the staphylococci is heat-resistant. Exposure to boiling temperatures for a moderate length of time does not materially decrease its potency, although heating for long periods in an autoclave destroys it. The toxin seems also to be resistant to proteolytic enzymes; treatment by trypsin and pepsin has been reported by many workers to have little effect upon the toxin. The toxin is resistant to alkali treatment at *p*H of from 8.0 to 8.2, but is less resistant to acid.

---

[34] W. B. Underwood, *Textbook of Sterilization*. American Sterilizer Co., Erie, 1934.

[35] G. M. Dack, *Food Poisoning*. Univ. of Chicago Press, Chicago, 1943.

[36] J. Stritar and E. O. Jordan, *J. Infectious Diseases*, **56**, 1 (1935).

The conditions which govern the production of the toxin are not well understood. Jordan and coworkers[37] are of the opinion that starch stimulates the production of toxin but we have ample evidence to indicate that the toxin can be formed in a food that contains no starch. There is some evidence that toxin is more readily produced under aerobic conditions than under anaerobic. That the composition of the medium has something to do with the ability of the organism to produce toxin is apparent from the fact that it is possible to grow potential toxin formers in a medium where no toxin is produced. Furthermore, it has been found that some toxin-producing strains lose their ability to form the toxin. This suggests the possibility that all staphylococci are potential toxin formers, and perhaps the only reason they do not always form toxin is that the environmental conditions are not right. Vigorous growth of the organism appears to be necessary for toxin formation, since food heavily contaminated with staphylococci will not become toxic if it is stored in an icebox. If it is left at room temperature for a few hours, however, it will be toxic. About all that can be said from our present state of knowledge is that any food that will support the growth of staphylococci is likely to become toxic if left at a favorable temperature.

Staphylococcic food poisoning is widespread and may occur at any time of the year, although more cases are reported during the summer than in the colder months. Many cases have been reported from bakers' products, such as custard-filled pie, custard-filled cakes, and eclairs. A number of outbreaks have been traced to dairy products, salad dressings made with butter and eggs, and to mildly cured hams. In nearly all cases, it has been found that the incriminating food had been allowed to stand at room temperature for several hours. In the cases that have been traced to ham, the hams had been left in warming ovens for a considerable time.

Symptoms of staphylococcic food poisoning may appear very soon after eating the contaminated food, within 3 to 6 hrs. The time probably depends upon the amount of toxin present and the amount of food consumed. Common symptoms are nausea, vomiting, abdominal cramps, and diarrhea. In severe cases, blood may be observed in the stools and vomitus. In mild cases, there may be nausea and vomiting but no diarrhea. Mortality is very low. Among the recorded cases, it falls below 1%, and since many cases are unrecorded the actual mortality is even lower. Individuals suffering from this poisoning recover within a few days, although in some cases the effects may be felt for more than a week. There is apparently great variation among individuals as to their susceptibility to the toxin. Human volunteers who are fed the same amount of toxin do not show the same symptomatology. Some will show no symptoms at all, others will be only mildly affected, and still others may have very severe symptoms. Usually the symptoms are severe enough so that it is not necessary to employ a stomach pump or to give cathartics. In some cases it may be necessary to treat the patient for loss of body fluids. There is no special drug or serum therapy that is of any value.

---

[37] E. O. Jordan and W. Burrows, *J. Infectious Diseases*, 57, 121 (1935).

Laboratory diagnosis of staphylococcic poisoning in suspected samples of food is frequently possible. Because the onset of symptoms is so rapid, it is very often possible to secure samples. A quantitative bacterial count should be made on the solid or liquid food involved. If the food is solid, it should be thoroughly ground with a mortar and pestle, using sand. In our own laboratories it has been found that the food can be broken up very effectively by means of a Waring blender. Quantitative plate counts should be made, using veal infusion agar. The plates should be counted at the end of 24 or 48 hrs. Blood agar and selective media such as eosin methylene blue or Endo's agar should also be used so as to rule out Gram-negative intestinal parasitic organisms such as those of the salmonella group. In the case of actual staphylococcic poisoning, it is usually possible to show that the staphylococci predominate, and the counts will show several hundred million per gram of food. So far as I know there have been no cases of staphylococcic food poisoning reported in which toxin was demonstrated in the absence of the organisms. Such cases might exist, but they would be very difficult to diagnose. If, in the analysis of the food, it is found that staphylococci predominate, that there are present several hundred millions of them per gram, and it is known that the patient had typical symptoms of staphylococcic food poisoning, one can be certain that the case is one of staphylococcic food poisoning. The finding of large numbers of staphylococci does not of itself constitute proof, however, since these organisms are so ubiquitous and foods are so universally contaminated with them.

Following the isolation of staphylococcus from the food, it would be desirable to determine whether the organism could produce toxin. At present there are no chemical tests for the toxin, and one must rely upon human volunteers or upon laboratory animals. Human volunteers are the best because human beings are more universally susceptible and the symptoms are more easily observed than in animals. Human volunteers are not always available, however, and it is usually necessary to depend on laboratory animals for the toxicity test. The animal most susceptible to the toxin is the rhesus monkey, but its cost makes its use almost prohibitive. Next to the monkey, a young cat or kitten seems to be best adapted for testing for the presence of this enterotoxin. It is necessary first to grow the organism in a medium that is known to favor toxin production. A filtrate from this medium is used for injection, and the animal is watched to see whether it develops symptoms of food poisoning. For detailed information on these tests, the reader is referred to the work of Dack and others.

The control of staphylococcic food poisoning is one of the important problems of the public health worker. The organisms are so widely distributed in nature that it is virtually impossible to prevent foods from becoming contaminated with them. Even though food could be handled in processing centers so that it was free of staphylococci, it would in all probability be contaminated by such organisms before it reached the hands of the consumer. When one combines this knowledge with a realization of the fact that many foods serve as excellent media for the growth of these organisms, one can see why it is very difficult to eliminate this type of food poisoning. The best method of control we have today is refrigeration.

Dack and coworkers have shown that if food is kept at 4 to 6° C., it will not develop the enterotoxin even though heavily contaminated by the food-poisoning staphylococci. The real problem in control is to educate the public as to the importance of refrigeration as a preventive measure. Dack summarizes the situation very aptly when he states that of all the factors involved in the control of staphylococcic food poisoning, refrigeration is paramount.

(c) *Salmonella Food Poisoning*

It was not many years ago that bacteriologists believed the salmonella group to be responsible for most cases of food poisoning. This was before Dack and his coworkers had clearly shown that staphylococci were implicated. In those days it was common practice to examine food for botulinus toxin, and if that could not be found, to assume that the poisoning was caused by *Salmonella*. This was often substantiated by isolating Gram-negative rods which would not ferment lactose, and which were then assumed to be members of the salmonella group and the cause of the poisoning. In many cases nothing further was done to determine whether these organisms were pathogenic, or to find out whether other organisms in the food could be implicated. In those cases where these organisms have been studied carefully, it has been found that some were not members of the salmonella group at all, but were slow lactose-fermenting "coli." When we consider the food-poisoning situation in the light of what is known today about staphylococci, we doubt that any but a very few of the earlier cases were caused by *Salmonella*. It is not to be inferred from this that *Salmonella* cannot cause intestinal disturbances that may be called food poisoning, because there are a few cases on record in which it appears that this organism has without question been incriminated.

It was commonly believed some years ago that *Salmonella* could cause food poisoning in very much the same way as we now know that staphylococci cause it. It was believed that the salmonella would elaborate a toxin, possibly an endotoxin, which, when ingested, would produce symptoms of food poisoning. In addition, it was known that the salmonella could cause an enteritis or an infection in the intestinal tract. Evidence obtained from a re-examination of the problem in recent years seems to indicate that all the so-called cases of Salmonella food poisoning are in reality cases of enteritis.

The salmonella group comprises a large number of closely related organisms. The ones that are known today by no means complete the list, since new ones are being described continually. As far as this problem is concerned they can be subdivided into two more or less distinct groups: one group that produces a disease in man similar to typhoid fever and another that produces disease in animals similar to typhoid fever in man. If a man consumes large numbers of the latter group, he may suffer from enteritis, with symptoms of nausea, vomiting, abdominal pain and diarrhea, frequently accompanied by high fever. Some of the strains that have been implicated are *Salmonella aërtrycke* and *Salmonella enteritidis*. In clear-cut cases of this kind which have been carefully studied, the organisms have been isolated not only from food but also from stools of the patient.[38] Furthermore, serum from the patient has agglutinated organisms isolated from the food and from the stool cultures. In the face of such evidence, one is forced to conclude that the organism isolated has been responsible for the symptoms observed. Several outbreaks of this so-called food poisoning have been reported in the literature. As early as 1888, Gaertner[39] studied an outbreak involving 57 persons, which was shown to be caused by meat from a diseased cow. This and similar outbreaks, as, for example, those described by van Ermengem,[40] show beyond a doubt that salmonella organisms can cause intestinal disturbances similar to those shown in typical food-poisoning cases.

There is one outstanding difference between salmonella intestinal infections and staphylococcic food poisonings, and that is the incubation period. Since the cases of so-called food poisoning caused by *Salmonella* are in reality food infections, it is easy to understand why there should be this difference. In the case of the salmonella, symptoms may appear anywhere from a few hours to several days after consuming the contaminated food. The onset is usually sudden. The duration is longer than in the case of staphylococcic food poisoning, and the recovery is not as rapid or complete. In case of death, the organisms have invaded the tissues. As in the case of staphylococcic food poisoning, the mortality is very low, probably less than 1%. In the diagnosis of salmonella food infection, the organism should be isolated from the food and also from the stools of the patient. This is sometimes very difficult. Because of the incubation period, it is often impossible to get samples of the food involved. In such cases, one will have to confine his investigation to the patient himself. If the symptoms are typical of the salmonella infection, if the organisms can be isolated from the stools, and if the patient's serum agglutinates the isolated organism, one has good grounds for a diagnosis of salmonella infection. It is unlikely that the patient became infected through any other source than food, since the known cases are all food-borne. Frequently in cases of this kind, the salmonella may replace the normal intestinal flora, and in such cases the organisms are found in great abundance in the stool culture. There are several good differential media available for the culture of these organisms, such as Endo's agar or eosin methylene blue agar. There are excellent dehydrated media also available. For details concerning the methods of isolation and identification of these organisms, the reader is referred to standard textbooks on bacteriology.

---

[38] F. W. Tanner, *Food-Borne Infections and Intoxications*. Garrard, Champaign, 1933.

[39] A. Gaertner, *Korresp. d. allg. ärztl. Vereins von Thüringen*, **17**, 233 (1888).

[40] E. van Ermengem, *Rev. hyg.*, **18**, 761 (1896).

One of the important methods of control of this food-borne enteritis is the rigid inspection of all animals that are slaughtered, since many of the carefully studied cases have been traced to animals that were suffering from an infection at the time of slaughter. With methods of inspection now employed in modern packing plants, such animals are detected and their flesh is condemned. This inspection service is under the administration of the Livestock and Meats Branch, Food Distribution Administration of the United States Department of Agriculture.

Salmonella capable of causing enteritis in man have frequently been isolated from the feces of rodents. Therefore another important step in the control of this disease is to see that food establishments, particularly meat storage places, are rat- and mice-proof. This, combined with rigid sanitation, the repeated sterilization of knives to prevent carrying contamination from one carcass to another, and the provision of an adequate pure water supply in slaughterhouses will keep this type of food infection very well in check. In addition to these methods of control, it is as important here, as in the control of staphylococcic food poisoning, to ensure that the meat is held under refrigeration from the time of slaughter until it is adequately cured or until it reaches the hands of the consumer. There is one additional precaution that should be exercised not only by the food processors, but also by consumers, and that is to prevent food from becoming contaminated by flies.

(*d*) *Other Organisms*

In addition to the organisms discussed above, there are a few other groups which have been considered responsible for or suspected of causing food poisoning. A few cases have been traced to the streptococci. In all such cases, the symptoms were similar to but milder than those caused by the staphylococci. These organisms are less resistant to chemicals and heat than the staphylococci.[41] Therefore, if foods are properly handled so as to avoid staphylococcic food poisoning, the control measures would be adequate for the control of poisoning from the streptococci as well.

There have been a number of cases reported in the literature where the investigators were of the opinion that still other organisms were involved—*Proteus*, *Escherichia*, and some strains of the *Pseudomonas*. It is highly probable that if food is heavily contaminated with any one of these groups, it may cause intestinal disturbances. Further evidence is needed, however, before we can be certain that the above-named organisms can actually be responsible for food poisoning.

---

[41] B. A. Linden, W. R. Turner, and C. Thom, *U. S. Pub. Health Repts.*, 41, 1647 (1926).

4. Protozoa, Helminths, and Nematodes

It is beyond the scope of this book to discuss in detail the illnesses caused by the ingestion of protozoa and parasites along with food. *Endamoeba histolytica*, which causes amebic dysentery, and *Trichomonas hominis*, which causes flagellate diarrhea, are protozoa. Other flagellates which may contaminate food are *Giardia lamblia* and *Chilomastix mesnili*. These flagellates live in milk and uncooked food and even in cooked food after it has cooked.

Trichinosis is a disease caused by the nematode, *Trichinella spiralis*, which lodges in the muscle fibers of hogs. Swine fed uncooked garbage are more likely to be infested than hogs fed on grain. Digestion of infested pork in the stomach sets free the larvae, which are excysted in the duodenum and invade the duodenal and jejunal mucosa. There they mature. The fertilized females bore through the wall of the intestine and deposit young trichinae, which pass into the blood stream and thus can be transported all over the body, particularly to the striated muscles, giving rise to symptoms mistaken for many other diseases. Fresh pork should be cooked at a temperature of 137° F. in all parts to make certain that the organism is killed. See page 463. Refrigeration can be used to kill the parasite.

Taenia saginata, the beef tapeworm, and *Taenia solium*, the pork tapeworm, are examples of helminths which also cause illness when ingested. *Trematoda* (including flukes) are other platyhelminths which cause illness.

Selected Bibliography

Buchanan, R. E., and Fulmer, E. I., *Physiology and Biochemistry of Bacteria*. Williams and Wilkins, Baltimore, 1930.

Dack, G. M., *Food Poisoning*. Univ. of Chicago Press, Chicago, 1943.

Damon, S. R., *Food Infections and Food Intoxications*. Williams and Wilkins, Baltimore, 1928.

Halvorson, H. O., and Ziegler, N. R., *Quantitative Bacteriology*. Burgess, Minneapolis, 1938.

Hammer, B. W., *Dairy Bacteriology*. Wiley, New York, 1928.

Henrici, A. T., *Molds, Yeasts, and Actinomycetes*. Wiley, New York, 1930.

Hull, T. G., *Diseases Transmitted from Animals to Man*. Thomas, Baltimore, 1941.

Jensen, L. B., *Microbiology of Meats*. Garrard, Champaign, 1942.

Jordan, E. O., *Food Poisoning and Food-Borne Infection*. Univ. of Chicago Press, Chicago, 1931.

Jordan, E. O., and Burrows, W., *Bacteriology*. Saunders, Philadelphia, 1941.

Knight, B. C. T. G., *Bacterial Nutrition*. Special Reports, Medical Research Council, London, 1936.

Savage, W. G., *Food Poisoning and Food Infections*. Cambridge Univ. Press, London, 1920.

Stephenson, M., *Bacterial Metabolism*. Longmans, Green, London, 1939.

Tanner, F. W., *Food-Borne Infections and Intoxications*. Garrard, Champaign, 1933.

Topley, W. W. C., and Wilson, G. S., *Principles of Bacteriology and Immunity*. Wood, Baltimore, 1937.

PART II

FOODS

Chapter XII

MILK, CREAM, AND DAIRY PRODUCTS

MORRIS B. JACOBS
Brooklyn, New York

I. MILK

In the general design of things, it was very likely meant that mammals should feed their young with their own milk. During the course of years and in accordance with the practice of the domestication of animals by human beings, it became customary for man to use the milk of these domesticated animals for food, the milk of cows and goats being the most used. In modern times the use of the milk of cows has far outstripped the use of any other milk.

TABLE 39

NUMBER OF MILK COWS ON FARMS, MILK AND BUTTERFAT PRODUCTION PER MILK COW, AND TOTAL QUANTITY OF MILK PRODUCED IN THE UNITED STATES, 1924–1941[a]

Year	Milk cows on farms, thousands[b]	Production per milk cow[c]		Total production of milk on farms, million lbs.
		Milk, lbs.	Butterfat, lbs.	
1924	21,417	4167	163.3	89,240
1925	21,503	4218	165.1	90,699
1926	21,312	4379	171.7	93,325
1927	21,191	4491	176.2	95,172
1928	21,223	4516	177.3	95,843
1929	21,618	4579	179.7	98,988
1930	22,218	4508	176.8	100,158
1931	23,108	4459	175.0	103,029
1932	24,105	4307	169.2	103,810
1933	25,062	4180	164.2	104,762
1934	25,198	4033	158.7	101,621
1935	24,187	4184	165.4	101,205
1936	23,727	4316	170.3	102,410
1937	23,340	4366	172.5	101,908
1938	23,215	4588	180.1	105,807
1939	23,273	4589	181.5	106,792
1940	23,684	4624	182.9	109,510
1941[d]	24,357	4742	187.5	115,498

[a] U. S. Dept. Agr., *Agricultural Statistics* (1942).

[b] Average number during year, heifers not freshened excluded. Includes allowances for seasonal variation and trend during the year.

[c] Excludes milk sucked by calves and milk produced by cows not on farms. To convert pounds of milk to gallons, divide by 8.6.

[d] Preliminary.

The origin of the use of milk of domesticated animals by man is not definitely known but animals were probably domesticated after 8000 B.C. Historical records show that milk was in common use as a food some 6000 years ago. The land we now call Palestine was depicted in the Bible as a land flowing with milk and honey in order to illustrate its value. It was only in the 18th and 19th centuries, however, that dairying as an industry was developed to any extent. For many years each family had its own cow but with the growth of large cities this custom became impractical and was soon superseded by the growth of dairies. At the beginning of the 19th century, about one-third of the milk used by London was supplied by cows kept in the city. With the development of refrigeration, modern means of transportation, and increase of sanitary regulations, the milk supply of large cities was obtained from dairies which had moved away from these cities and were located at times far from the metropolitan area.

TABLE 40

MANUFACTURED DAIRY PRODUCTS: PRODUCTION PER CAPITA, SELECTED PERIODS, 1870–1937[a]

Period	Butter, lbs.[b]	Cheese, lbs.[b]	Condensed milk, lbs.[c]	Evaporated milk, lbs.[d]	Commercial ice cream, gal.	Malted milk, lbs.	Dried whole milk, lbs.	Dried cream, lbs.	Total milk equivalent, lbs.
1910–1914	17.6	3.82	7.45		0.545	0.046	0.018	0.0015	431
1924–1929	17.7	4.06	2.67	10.86	1.978	0.171	0.088	0.0043	466
1870–1879	13.4	4.94	0.18		0.002	.....	.....	......	331
1880–1889	16.4	4.93	0.48		0.008	.....	.....	......	395
1890–1899	18.5	4.09	1.52		0.036	.....	.....	......	434
1900–1909	18.8	3.85	3.96		0.177	0.006	0.001	0.0001	444
1910–1919	16.9	4.13	10.66		0.838	0.087	0.038	0.0032	431
1920–1929	17.2	4.05	2.88	10.38	1.816	0.162	0.077	0.0034	453
1930–1937	17.5	4.46	1.79	13.51	1.638	0.134	0.119	0.0011	467

Percentage Each Product Is of Total Milk Equivalent

Period	Butter, %[b]	Cheese, %[b]	Condensed milk, %[c]	Evaporated milk, %[d]	Commercial ice cream, %	Malted milk, %	Dried whole milk, %	Dried cream, %	Total milk equivalent, %
1910–1914	85.436	8.890	3.694		1.911	0.028	0.034	0.007	100.0
1924–1929	78.680	8.724	0.833	5.130	6.369	0.096	0.150	0.018	100.0
1870–1879	84.821	15.056	0.115		0.008	.....	.....	.....	100.0
1880–1889	87.149	12.560	0.263		0.028	.....	.....	.....	100.0
1890–1899	89.562	9.560	0.754		0.124	.....	.....	.....	100.0
1900–1909	88.801	8.657	1.933		0.604	0.004	0.001	[e]	100.0
1910–1919	82.030	9.597	5.290		2.945	0.053	0.071	0.014	100.0
1920–1929	78.724	8.978	0.993	5.058	6.004	0.093	0.136	0.014	100.0
1930–1937	78.034	9.556	0.484	6.369	5.274	0.074	0.204	0.005	100.0

[a] E. E. Vial, U. S. Dept. Agr., *Tech. Bull.* 722 (1940).

[b] Farm and factory.

[c] Bulk and case, unskimmed, including unsweetened condensed milk.

[d] Case unskimmed.

[e] Less than 0.0005%.

The first real cheese factory in the United States is reported to have been built in New York State in 1851 and the first creamery was developed during the War between the States. Since then the growth of the dairy industry has been phenomenal for dairy products are the largest source of agricultural income.[1] It was equivalent to 16–17% or $1,700,000,000 of cash farm income in 1939.

TABLE 41

MANUFACTURED DAIRY PRODUCTS: CONSUMPTION PER CAPITA, SELECTED PERIODS, 1870–1937[a]

Period	Butter, lbs [b]	Cheese, lbs.[b]	Condensed milk, lbs [c]	Evaporated milk, lbs [d]	Commercial ice cream, gal	Malted milk, lbs [e]	Dried whole milk, lbs [e]	Dried cream, lbs.[e]	Total milk equivalent lbs.
1910–1914	17 5	4 28	7 27		0 545	0 046	0 018	0 0015	434
1924–1929	17 6	4 60	2 28	9 93	1 978	0 151	0 082	0 0045	467
1870–1879	13 1	2 83	0 18		0 002				305
1880–1889	16 1	3 13	0 48		0 008				370
1890–1899	18 2	3 33	1 52		0 036				421
1900–1909	18 6	3 89	3 96		0 177	0 006	0 001	0 0001	441
1910–1919	16 8	4 22	8 51		0 838	0 087	0 038	0 0032	424
1920–1929	17 1	4 46	2 23	9 18	1 816	0 146	0 063	0 0035	453
1930–1937	17 6	4 86	1 71	13 09	1 638	0 114	0 105	0 0012	472

Percentage Each Product Is of Total Milk Equivalent

Period	Butter, %[b]	Cheese %[b]	Con densed milk, % [c]	Evaporated milk %[d]	Commercial ice cream %	Malted milk, %[e]	Dried whole milk, %[e]	Dried cream, %[e]	Total milk equivalent, %
1910–1914	84 581	9 881	3 574		1 895	0 028	0 034	0 007	100 0
1924–1929	78 224	9 844	0 646	4 685	6 359	0 084	0 140	0 018	100 0
1870–1879	90 429	9 438	0 125		0 008				100 0
1880–1889	91 194	8 496	0 280		0 030				100 0
1890–1899	91 099	7 998	0 777		0 126				100.0
1900–1909	88 617	8 827	1 945		0 606	0 004	0 001	[f]	100.0
1910–1919	82 611	9 969	4 252		3 025	0 055	0 073	0 015	100.0
1920–1929	78 793	9 860	0 678	4 452	6 008	0 084	0 111	0 014	100 0
1930–1937	77 625	10 333	0 454	6 116	5 226	0 063	0 178	0 005	100 0

[a] E E Vial, U S Dept Agr , *Tech Bull* 722 (1940).
[b] Farm and factory
[c] Bulk and case, unskimmed, including unsweetened condensed milk
[d] Case, unskimmed
[e] Where consumption was not available, production was used
[f] Less than 0 0005%

Milk and cream alone, or as milk products, or in combination with other foods, comprise about one-sixth of the weight of food eaten by an average American family. About 40% of the milk produced in the United States is used as fluid milk and cream while about half is utilized in the manufacture of dairy products. The production and consumption per capita of manufactured dairy products for selected periods from 1870 to 1937 is given in Tables 40 and 41. The reader is referred to the U. S. Dept. Agriculture publication, *Agricultural Statistics*, for more complete data. There has been a marked increase in the utilization of milk for industrial purposes. Some of the by-products of milk are casein, casein paint, lactose, lactic acid and its salts. These products are used in many industries.

---

[1] E E Vial, U. S Dept Agr , *Tech. Bull* 722 (1940).

While milk is the normal secretion of the mammary glands of mammals, it is generally defined as the whole, fresh lacteal secretion obtained by the complete milking of one or more healthy cows, excluding that obtained within 15 days before and 5 days after calving, or such longer period as may be necessary to render the milk practically colostrum-free. As is clear from this definition, the term milk, unqualified, means cow's milk. All other milk is qualified by some adjective such as human milk, goat's milk, etc.

1. Physical Properties of Milk

Milk is a complex physicochemical system for it is a mixture consisting of a solution of salts, lactose, and lactalbumin in water, in which solution, fat and some minor lipid components are emulsified, and in which proteins and calcium phosphate are colloidally dispersed. Consequently the physical and chemical properties of milk are functions of its components.

(a) *Appearance, Odor, and Taste*

Milk has a characteristic appearance, odor, and taste. These factors are conditioned by the feed given the cow. It was formerly considered that off-flavors were absorbed by the milk after it was drawn from the cow but it has been shown that they are transmitted principally by the cow itself. The normal white or faint ivory tone of milk is attributed to the light reflected from the colloidally dispersed fat and protein. A bluish tint is ascribed either to dilution of milk with water or to skimming. While the taste of milk is characteristic it is not pronounced. A marked variation from the slightly sweet taste of normal milk may be considered abnormal.

(b) *Specific Gravity*

Since milk contains many components whose density is greater than that of water, its specific gravity will be greater than that of water. Milk normally has a specific gravity which varies in the range 1.027–1.035 with an average of 1.032 at 60° F. The average, however, upon which the Quevenne and New York Board of Health lactometers are based is 1.029 at 60° F. Milk is a fat-in-water emulsion, consequently its specific gravity will be a function of the specific gravity of the fat and of the water solution. The specific gravity of the fat is about 0.93, that of the water solution is about 1.035; hence as the fat content of the milk increases, the specific gravity decreases and, conversely, as the solids-not-fat increase, the specific gravity of the milk increases. The actual specific gravity is some function of the two.

(*c*) *Freezing Point*

A property depending entirely on the number of molecules present is termed a colligative property. Such colligative properties for substances in solution are vapor pressure, osmotic pressure, elevation of boiling point, depression of freezing point, and others. Van't Hoff showed that these properties were all interrelated. Since the freezing-point depression is dependent on the number of dissolved particles, then quantities proportional to the molecular weight, *i. e.*, quantities containing the same number of molecules, dissolved in identical weights of a solvent, will cause equal depressions of the freezing point of that solvent.

It is known that the osmotic pressures of body fluids, such as blood and milk, are almost constant. Hence the freezing points of these fluids are also almost constant. This constant freezing point in milk is due to the combined effects of three of the soluble components of milk, namely, lactose, the salts of potassium and sodium, and the salts of calcium and magnesium. The smallest osmotic pressure of genuine milk ever observed is represented by a freezing-point depression of about 0.53° C. (0.95° F.), as determined by the Hortvet method.[2, 3] The average depression obtained by different observers varies from 0.54 to 0.55° C. If, therefore, a fresh milk gives a freezing-point depression of less than 0.53° C., *i. e.*, its freezing point is higher than −0.53° C. (31.05° F.), it is definite proof of the presence of added water.

(*d*) *Boiling Point*

A characteristic property of a solution is the elevation of the boiling point. Since milk is a solution, its boiling point is higher than that of water with an average value of 100.17° C. (212.3° F.).

(*e*) *Viscosity and Fluidity*

The viscosity of a fluid is the internal friction which tends to bring to rest portions of the fluid which are moving relative to one another. It is the resistance which particles of a fluid offer to free motion one over the other, and is measured in relation to some standard viscosity, generally water at 25° C.

The fluidity, which is the reciprocal of the viscosity of a suspension, varies as the volume of the solid component to the total volume of the suspension. If the number or the size of the solid particles increases, a type of structure will finally be formed which will impede flow. This is the point where plastic flow begins and viscous flow ends. Variations in temperature have an effect on the volume of the suspended phase as well as on the fat emulsion. Below its melting point a fat has the properties of a plastic solid, whereas above its melting point the fluidity of a fat is the factor concerned.

The viscosity of milk decreases and, conversely, the fluidity of milk increases, with

---

[2] M. B. Jacobs, *Chemical Analysis of Foods and Food Products*. Van Nostrand, New York, 1938.

[3] J. Hortvet, *Ind. Eng. Chem.*, **13**, 198 (1921).

an increase in temperature, but this function is not a straight-line relationship. The viscosity of milk ranges from 4.28 at 0° C. to 1.64 at 30° C. Based on the fluidity of water as 1.0 at 20° C., the fluidity of milk ranges from 0.233 at 0° C. to 0.609 at 30° C., whereas that of water is 0.558 at 0° C. and 1.25 at 30° C.

With increase of the aggregation of the fat particles as in the case of cream, there is an increase in the viscosity. Increasing the dispersion of the fat by homogenization increases the viscosity. Heating milk to temperatures used in pasteurization decreases its viscosity. Prolonged shaking increases its fluidity, while aging and souring decrease its fluidity.

(*f*) *Refractive Index*

The refractive index of a solution is the total of the refraction of light caused by each of the dissolved substances and the solvent itself, *i. e.*, it is an additive function of the molecular concentration of the solutes and the solvent. The refractive index of milk ranges from 1.3470 to 1.3515 according to Jorgensen.[4] However, since the refractive index is a function of the dissolved substances, it is generally obtained on milk serum. This is a lower value and ranges from 1.3430 to 1.3443 probably because of the absence of soluble protein.

(*g*) *Conductivity*

The conductivity of milk is a function of its ionic components. The fluidity of the milk, however, and the obstruction of the movement of the ions by colloidally dispersed particles reduce its conductivity, hence the removal of fat increases its conductivity. Because of the variation in its ionic components, the conductivity of milk may vary over a wide range[5] from 38.0 to 68.4 $\times 10^{-4}$ mhos, with some investigators giving a wider range, but the average for healthy cows probably falls in a range from 45 to 50 $\times 10^{-4}$ mhos. Diseased cows yielding milk with unusually high salt content, or the presence of neutralizers, will increase the conductivity because of the increase in ions.

(*h*) *Specific Heat*

The specific heat of a substance is the ratio of its thermal capacity to that of water at 15° C. It is the ratio of the amount of heat necessary to raise a given weight of a substance to a given temperature, to the amount of heat required to raise an equal weight of water to the same temperature. The density of a substance affects its specific heat. The specific heat of milk is an additive function of its components but is affected by the latent heat of fusion of the fat. Milk, skim milk, and whey have lower specific heats than water but there is a relatively straight line relationship with a hump extending over 20 degrees, its peak being at about 20° C. because of the melting of the fat. The specific heat of milk is 0.920 at 0° C., 0.938 at 15° C., 0.930 at 40° C., and 0.918 at 60° C. Cream and butter, because of the low specific heat of fat which is of the order of 0.5, will have lower specific heats than milk but at the melting point of the fat the latent heat of fusion of the fat may increase the specific heat of cream even above that of water. Thus at 15° C. 45% cream has a specific heat of 1.016 and 60% cream has a specific heat of 1.053. These values rise sharply from about 0.6 at 0° C., then fall sharply to about 0.7 at 40° C.

---

[4] A. Jorgensen, *Landw. Jahrb.*, **11**, 701 (1882).

[5] J. Krenn, *Milchw. Forsch.*, **14**, 513 (1933).

(*i*) *Surface Tension*

The surface particles on any given liquid have greater molecular forces tending to pull these particles toward the interior of the liquid rather than outward. Hence, a fluid acts like a stretched elastic membrane. This effect is called surface tension. In milk, the surface tension is influenced by the presence of proteins, fat, and salts. The surface tension of milk as measured by the Du Noüy ring method is of the order of 50 dynes per sq. cm.;[6] that of skim milk is somewhat higher, of the order of 55 dynes per sq. cm. Increasing the amount of fat or development of acidity decreases the surface tension, while increasing the amount of air increases the surface tension.

(*j*) *Curd Tension*

The resistance exhibited by a curd produced by the action of pepsin on milk to the passage of a knife through it is known as the curd tension. There are a number of processes which are used to alter the properties of curd in milk so as to make the milk more digestible. Most milk has a curd tension varying from 60 to 250 g.; that is, there is a resistance equivalent to that weight in pulling the Hill[7, 8] curd knife through the curd. A soft curd milk such as a homogenized milk will have a curd tension which varies from 0 to 30 g.

(*k*) *Size of Fat Globules*

The size of fat globules in fresh milk which has not been subjected to agitation varies from 0.1 to 10 μ, the average being in the range 2.5–3 μ. The number of globules ranges from about 2.5 to 5×10^9 per ml. Normal milk of high fat content contains a greater number of larger globules than normal milk of low fat content. The principal factors in the variation of size are the breed of the animal and the stage of lactation. Jersey and Guernsey cows produce larger globules than Ayrshire and Holstein breeds. Goat milk contains about 9×10^9 globules per ml. of smaller size than cow's milk. As the lactation period continues, the size of the globules becomes smaller. Cream containing large fat globules churns more easily than cream composed of small fat globules. The smaller the size of the fat particles, the larger the loss in the skim milk in the period before the centrifugal cream separator was extensively used.

---

[6] J. A. Gamble, N. R. Ellis, and A. K. Besley, U. S. Dept. Agr., *Tech. Bull.* **671** (1939).

[7] R. L. Hill, *J. Dairy Sci.*, **6**, 509 (1923).

[8] H. E. Otting and J. J. Quilligan, *Milk Dealer*, **23**, 36 (1934).

(*l*) *Creaming*

Roadhouse and Henderson[9] state that no fully acceptable theory to explain the creaming of milk has been proposed. Since the lipid fraction of milk has a lower specific gravity than the milk serum, the fat will tend to rise. If this rise is to follow Stokes' law, some clumping of the fat, which will be controlled by the milk serum, should occur. Lactoglobulin may be the substance in milk serum which has this effect. It is possible that the charge on the fat globules contributes to the clumping action but this factor is less important than the component or components in the serum which control clumping.

(*m*) *Other Physical Properties*

Some other physical properties of milk which are important at times are those of adhesion, wetting power, and coefficient of expansion. Because it contains casein, milk exhibits adhesive properties. The adhesiveness of milk increases with decrease in temperature, consequently it is more difficult to wash out a vessel which held cold milk than one which contained warm milk. The wetting power of a material depends not only on its surface tension but also on its intrinsic nature and on its relation to the composition and properties of the material wetted. Water has less wetting power than milk, probably because the milk contains casein.

Milk, like water above 4° C., expands when heated and contracts when cooled. This property is of considerable importance in the filling of containers with milk and when milk is bought on a volume basis.

The nature of milk foam is discussed on page 46.

2. Physicochemical Properties of Milk

It has been stressed in a previous section that milk is a most complex physicochemical system because of its composition. It is a heterogeneous mixture of ions of strong salts, ions of weak salts, the salts themselves, ampholytes such as proteins, and nonelectrolytes such as fats, and some minor lipid components. The principal physicochemical factors to be considered are the acid-base equilibria, namely, *p*H, buffer capacity, and titratable acidity.

(*a*) *Hydrogen-Ion Concentration*

When milk is freshly drawn from the cow it is amphoteric to litmus paper for it will turn red litmus paper blue and blue litmus paper red. This is attributed to the proteins contained in milk which function as amphoteric substances at the normal *p*H of milk. This is about 6.6. Normal milk, however, may vary in *p*H from 6.3 to 6.7.

---

[9] C. L. Roadhouse and J. L. Henderson, *The Market-Milk Industry.* McGraw-Hill, New York, 1941.

(*b*) *Buffer Capacity*

The proteins, citrates, bicarbonates, and phosphates of milk form the principal buffer systems of milk. The buffer index is defined as the number of gram-equivalents of acid or base required to change the *p*H of one liter of buffer solution one unit of *p*H. Mathematically stated,

$$\text{buffer index} = \frac{dB}{dp\text{H}} = \frac{\text{ml.} \times N}{\text{vol. milk} \times p\text{H change}}$$

The buffer index of milk at *p*H 6.6 is about 0.0186. The index increases with decrease in *p*H but decreases again at *p*H 5.5 (see Fig. 27). The buffer capacity of milk is greater on the acid side than on the alkaline side of the *p*H scale. It decreases with increase of *p*H and has a sharp break at about *p*H 8.5 on further addition of alkali.

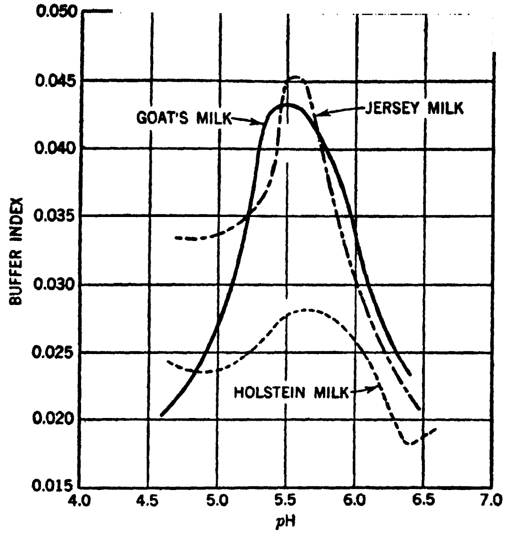


Fig. 27.—Buffer capacity of milk at different hydrogen-ion concentrations.[6]

(*c*) *Titratable Acidity*

Because the buffer capacity of milk is so small at *p*H 8.5 and because phenolphthalein undergoes a change in that *p*H region, that indicator can be used to estimate the so-called acidity of milk. Actually what is measured is the amount of alkali necessary to add to elevate the *p*H from the normal of 6.6 to the *p*H at which phenolphthalein indicator changes. The number of milliliters of 0.1 *N* sodium hydroxide required to bring about this change in 17.6 ml. of milk divided by 20 gives the percentage of acid in terms of lactic acid. It must be understood that this is a convention and does not mean that that much lactic acid is present. Normal milk has a titratable acidity which averages about 0.15–0.16%. The range for normal milk from individual cows extends from 0.10 to 0.22% but normal commercial fluid milk practically never exceeds the average value. The titratable acidity of milk is largely due to the casein (0.05–0.08%) and phosphate content.[10] Carbon dioxide (0.01–0.02%), citrates (0.01%), and albumin (less than 0.01%) also contribute to it.

(*d*) *Coagulation*

Milk coagulates naturally through the action of the enzyme rennin or because of the lactic acid produced by lactose-fermenting organisms. Heat is also a factor which causes coagulation. Two factors are involved in the coagulation of milk by heat. One is the chemical denaturation of the proteins and the other is the physical agglutination of the proteins. The formation of a skin by milk when heated is a phenomenon of coagulation.

3. Composition of Milk

Milk is one of the most important foods in the human diet because it has many components present in very small quantities that are essential to growth and well-being. That this is true follows from the fact that mammalian animals can live and thrive for weeks and months without the addition of other foods. There are other foods which contain these materials also but are not so easily assimilable as milk.

TABLE 42

COMPOSITION OF MILK, %

Analysis	Maximum	Minimum	Average	N. Y. State	Andrade
Water	90.0	82.0	87.3	87.1	87.0
Fat	7.8	2.3	3.67	3.9	4.2
Casein	....	1.5	2.86	2.5	...
Albumin	....	0.5	0.56	0.7	...
Total protein	4.5	2.0	3.42	....	3.27
Lactose	6.0	3.5	4.78	5.1	4.78
Ash	0.9	0.6	0.73	0.7	0.7
Total solids	18.0	10.0	12.69	12.9	12.98
Solids-not-fat	10.6	7.5	8.77	9.0	8.77

Milk consists largely of water, milk fat, lactose or milk sugar, protein, and mineral matter. These are probably present in some form of combination such as fat-protein, protein-salt, and similar combinations. The three main characteristic components are milk or butterfat, casein, and lactose.

William Prout[11] pointed out more than 100 years ago that milk is designed by nature expressly as food and that whatever the source, it "is a mixture of three stami-

---

[10] F. R. Rice and A. L. Markley, *J. Dairy Sci.*, **7**, 468 (1924).

[11] W. Prout, *Chemistry, Meteorology and the Function of Digestion Considered with Reference to Natural Theology*. London, 1834.

TABLE 43
COMPOSITION OF MILK

Fraction	Component
Lipid	Consists of fat composed of the mixed triglycerides of the following fatty acids in order of importance: oleic, palmitic, myristic, stearic, and butyric; of lesser importance—caproic, caprylic, lauric, capric, decenoic, tetradecenoic, hexadecenoic, and arachidonic. In addition, the oil-soluble vitamins, cholesterol, xanthophyll, phospholipids, cephalin, and lecithin are present
Protein	Casein, lactalbumin, and lactoglobulin. These proteins contain some 20 or more amino acids including all of the essential ones
Carbohydrate	Lactose or milk sugar
Mineral	Sodium, potassium, calcium, and phosphorus, chlorine, and sulfur in substantial quantities. Magnesium, copper, iron, zinc, manganese, and iodine in smaller amounts. Carbonates are also present
Vitamin	Vitamin A and its precursors such as carotene, thiamine, ascorbic acid, vitamins D, vitamins E, riboflavin, niacin, pantothenic acid, and pyridoxine are present
Enzyme	Phosphatase, amylase, lipase, catalase, peroxidase, galactase, reductase
Other organic	Citric and lactic acid, creatine, creatinine, urea, choline
Gas	Carbon dioxide, oxygen, and nitrogen
Water	

TABLE 44
AVERAGE MONTHLY COMPOSITION OF HOLSTEIN AND JERSEY COW'S MILKS (1929–1932)[a]

Month[b]	Fat, %		Protein, %		Lactose, %		Ash, %		Water, %	
	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey
February	3.8	5.1	3.19	3.77	4.53	4.46	0.71	0.71	87.90	85.85
March	3.7	5.4	3.09	3.72	4.58	4.65	0.81	0.73	87.98	85.68
April	3.7	5.5	3.08	3.65	4.68	4.93	0.70	0.70	87.96	85.57
May	3.8	5.4	3.16	3.72	4.76	4.90	0.68	0.70	87.93	85.66
June	3.2	5.5	3.14	3.72	4.44	4.62	0.68	0.70	88.29	85.71
July	3.4	5.4	3.10	3.73	4.54	4.58	0.69	0.70	88.49	86.05
August	3.5	5.2	3.06	3.66	4.34	4.59	0.69	0.73	88.66	86.09
September	3.2	5.0	3.19	3.89	4.56	4.86	0.69	0.72	88.77	85.86
October	3.5	5.5	3.18	3.86	4.70	4.77	0.72	0.74	88.05	85.50
November	3.6	5.2	3.30	4.08	4.50	4.65	0.70	0.76	88.07	85.61
December	3.5	5.5	3.34	4.30	4.88	4.96	0.74	0.79	88.00	84.74
Av.	3.4	5.3	3.17	3.83	4.59	4.73	0.70	0.73	88.19	85.67

Month	Iron, mg.[c]		Copper, mg.[c]		Calcium, mg.[c]		Phosphorus, mg.[c]	
	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey	Holstein	Jersey
February	.....	.....	0.060	0.055	112	121	95	97
March	.....	.....	0.070	0.055	117	127	91	112
April	0.06	0.09	0.047	0.053	107	119	91	101
May	0.077	0.09	0.058	0.055	97	116	94	101
June	0.075	0.11	0.054	0.048	107	116	89	98
July	0.062	0.12	0.053	0.060	101	104	87	89
August	0.08	0.07	0.064	0.069	102	115	79	88
September	0.065	0.075	0.056	0.060	107	122	84	94
October	0.08	0.08	0.057	0.070	99	113	94	123
December	0.080	0.075	.....	.....	111	...	84	113
Av.	0.072	0.088	0.057	0.058	106	117	88	101

[a] J. A. Gamble, N. R. Ellis, and A. K. Besley, U. S. Dept. Agr., *Tech. Bull.* 671 (1939).

[b] Analyses of water, protein, lactose, fat, ash, and total nutrients were made from February to December, 1929–1931, which includes, in addition, February to August, 1932. Iron analyses were made in 1929–1930, copper from April, 1931, to March, 1932, and calcium and phosphorus in 1929–1931. Except for copper, averages do not include data for 1932.

[c] Results are expressed as milligrams per 100 ml. of milk.

nal principles we have described; in other words, milk always contains a saccharine principle, a butyraceous or oily principle, and a caseous, or, strictly speaking, an albuminous principle."

Among the minor components, but nevertheless very important, are lactalbumin, lactoglobulin, lactic acid, the sodium, potassium, calcium, and magnesium chlorides, phosphates and citrates, iodine, cholesterol, lecithin, enzymes and the vitamins A, B_1, B_2, and C.

Davies[12] gives as the proximate composition of milk, based on the analysis of thousands of samples by various investigators of the United States, England, Scotland, and other countries: fat, 3.71%; solids-not-fat, 8.99%; and total solids, 12.7%. The maximum, minimum, and average percentage compositions of the more important components of milk as quoted by Jacobs[13] from various sources, average values obtained by the New York State Agricultural Experiment Station at Geneva, and the average values cited by Andrade[14] for the composition of milk of different breeds are given in Table 42.

A more complete picture of the composition of milk can be obtained from Table 43.

The average monthly composition of the milk of Holstein and Jersey cows is given in Table 44.

(*a*) *Lipid Fraction*

The lipid fraction of milk is a very complex mixture itself. The major component is milk or butterfat. The composition of butterfat is discussed on page 423. Oleic acid, followed closely by palmitic acid, is the predominant fatty acid. Myristic, stearic, butyric, capric, and lauric acids follow in order of importance. Those which appear in relatively small amounts or only in traces are caproic, caprylic, decenoic, tetradecenoic, hexadecenoic, and arachidonic acids. Included in this group are the fat-soluble substances such as the precursors of vitamin A, the vitamins D, cholesterol, phospholipids, cephalin, and lecithin.

(*b*) *Protein Fraction*

The protein fraction of milk consists principally of casein and lactalbumin. The latter comprises about 0.5% of the protein content of milk and casein comprises practically all of the remaining portion of the protein content. Lactoglobulin seldom exceeds 0.1% of the protein content of milk. There are said to be traces of fibrin and an alcohol-soluble protein.

(*c*) *Carbohydrate Fraction*

The sole carbohydrate isolated from milk is lactose. The chemistry of this compound is discussed on pages 64 and 789. Cow's milk generally contains about

---

[12] W. L. Davies, *Chemistry of Milk*. Van Nostrand, New York, 1936.

[13] M. B. Jacobs, *Chemical Analysis of Foods and Food Products*. Van Nostrand, New York, 1938.

[14] J. L. Andrade, *Estudios sobre la leche*. Caracas, Venezuela, 1940.

4.8% of lactose but human milk contains much more, of the order of 6.8–7.0% as noted on page 412.

(*d*) *Mineral Fraction*

The ash of milk is one of its most constant factors. The minimum recorded for normal milk is 0.6%, the maximum is 0.9%, and the average 0.7%. The vast majority of milks, however, have an ash content ranging from 0.68 to 0.74%.

The exact composition of the mineral components of milk has never been evaluated. Table 45 gives some values for the composition of the ash of milk. It can be seen from this table that potassium, sodium, calcium, and magnesium are the more important metallic constituents of the ash, while phosphorus, chlorine, and sulfur are the more important nonmetallic constituents.

TABLE 45

THE COMPOSITION OF THE ASH OF MILK[a]

Mineral	Ranges of values found by several European investigators, %	Values reported by Babcock, %	Mineral	Ranges of values found by several European investigators, %	Values reported by Babcock, %
Potassium	14.6 –23.9	20.8	Iron	0.035– 0.280	0.091
Calcium	14.2 –20.5	14.3	Phosphorus	9.44 –12.8	10.6
Sodium	1.93– 8.2	7.4	Chlorine	12.2 –16.4	14.3
Magnesium	0.72– 3.01	1.46	Sulfur	1.53 – 2.46	2.30

[a] P. A. Wright, E. F. Deysher, and C. A. Cary, U. S. Dept. Agr., *Yearbook, Separate No.* **1704** **(1940)**.

In addition to the mineral elements noted, milk contains small amounts of copper, iron, zinc, manganese, aluminum, and iodine. The quantities of some of these are very small but they are very necessary for adequate nutrition as has been stressed on page 270. Milk contains about 0.15 p. p. m. of copper, from 1 to 4.5 with an average of 2.5 p. p. m. of iron, about 3.6 to 4.12 p. p. m. of zinc, about 0.02 to 0.06 p. p. m. of manganese, about 15.4 p. p. m. of aluminum, and about 0.001 to 0.275 p. p. m. of iodine.

(*e*) *Vitamin Fraction*

The vitamin content of raw milk is variable. It depends chiefly upon the kind and quality of the feed given to the cow and possibly upon the amount of sunlight to which the cow is exposed. The available data have been summarized by Daniel and Munsell[15] and are discussed by Cary.[16] Cows which are well fed or are pasture fed generally produce milk which is rich in vitamin A. Milk is a fair source of vitamin B_1 and an excellent source of vitamin B_2. Other vitamins of the B complex such as niacin, pantothenic acid, and pyridoxine have been shown to be present. Raw milk is a fair source of vitamin C but this factor is little affected by the feed. Milk is a poor source of vitamin D. This factor can be increased by feeding irradiated yeast or ergosterol but other methods of fortifying milk with vitamin D are used (see page 415). Milk normally contains only a small amount of vitamin E. A digest of some of the known vitamin contents of milk is given in Table 46.

---

[15] E. P. Daniel and H. E. Munsell, U. S. Dept. Agr., *Misc. Pub.* **275** (1937).

[16] C. H. Cary, "Food and Life," U. S. Dept. Agr., *Yearbook*, **1939**, p. 668.

TABLE 46

VITAMIN CONTENT OF MILK[a]

Type	Units[b] per 100 g. of				
	A	B_1	B_2	C	D
Whole milk, av. market	165	20	60	0	2
From dry feed	80	20	30	1	1
Pasture fed	250	20	90	3	4
Dried	...	150	500	0	..
Dry feed	650	..	..	..	..
Pasture	2000	..	.	..	..
Skim milk	2	..	..	..	..
Dried	20	180	625	..	..

[a] E. P. Daniel and H. E. Munsell, U. S. Dept. Agr., *Misc. Pub.* 275 (1937).

[b] Values for vitamins A, B_1, B_2, and C are in Sherman Units, vitamin D is given in International Units.

(*f*) *Enzyme Fraction*

The principal enzymes present in milk are amylase, catalase, peroxidase, lipase, and phosphatase. Galactase, lactase, and aldehydase are also said to be present. The chemistry of the enzymes is discussed on page 185. The activity of lipase may cause off-flavors in milk. The presence or absence of phosphatase has been used as a test for the efficacy of pasteurization.[17]

(*g*) *Other Components*

A characteristic component of milk is citric acid which is present in concentrations of the order of about 0.01%. Carbon dioxide, oxygen, and nitrogen are also present. Other components such as creatine, choline, urea, and trimethylamine are present only in traces. Choline is probably derived from lecithin which hydrolyzes to form glycerophosphoric acid and choline. Marquez has suggested the use of the choline test as a measure of milk quality.[18]

If care is taken in production, handling, and processing, milk will have few bacteria present, the average number being about 500 per cc. in milk from the interior of the cow's udder. Most milk is exposed to some contamination from the outside during the milking process, consequently bacterial counts are higher. Freshly drawn milk produced under clean conditions will generally have counts under 3000, whereas freshly drawn milk produced under dirty conditions will have counts generally over

---

[17] M. B. Jacobs, *Chemical Analysis of Foods and Food Products.* Van Nostrand, New York, 1938.

[18] V. M. Marquez, *Variation Found in the Choline Contents of Milk during Its Aging.* Johns Hopkins University School of Public Health, Baltimore, 1942.

10,000 and ranging up to over 2,000,000. The bacteria get into the milk from the body and udder of the cow, from the hands of the milker or from the milking machine, from the utensils, from the barn, and during the subsequent handling and processing of the milk. It is clear, therefore, that scrupulous cleanliness must be observed in order to produce milk with low bacterial counts.

In addition to bacteria, body cells, yeasts, and molds are found in milk occasionally (see Vol. II, Chapter XX). These are seldom of importance.

(*h*) *Variation in the Composition of Milk*

The composition of milk varies with the breed of cow (as illustrated in Table 47), the time of year, the time of day, the portions of any one milking, the individuality of the cow, the age of the cow, the period of lactation, the type of feed, the length of interval between milkings, and other factors. The differences due to breed, however, are of the greatest magnitude. Thus Jersey, Guernsey, and Ayrshire breeds give milk which is richer in fat than Holstein and Shorthorn. Guernsey and Jersey breeds yield milk having greater total solids than that of Ayrshire, Holstein, and Shorthorn. In the fall and early winter richer milk is obtained than in the spring and early summer. The evening milking yields richer milk than the morning milking. The first portions ("fore" milk) of milk drawn in the milking process are poorer than the last portions or "strippings." It is very difficult to change the chemical composition of milk permanently by feeding. A temporary increase in the fat content can be obtained by feeding large quantities of fat. Iodine is the only inorganic component of milk that has been varied successfully by feeding.

TABLE 47

VARIATION IN COMPOSITION OF MILK DUE TO BREED[a,b,c]

Breed	No. of cows	Fat, %	Protein, %	Lactose, %	Ash, %	Total solids, %	Water, %	Solids-not-fat, %	Ratio of fat to solids-not-fat, %
Jersey	29,495	5.37	3.92	4.93	0.71	14.91	85.09	9.54	1:1.78
Guernsey	32,562	4.95	3.91	4.93	0.74	14.61	85.39	9.66	1:1.97
Brown Swiss	721	4.01	3.61	5.04	0.73	13.41	86.59	9.40	1:2.34
Ayrshire	6,999	4.00	3.58	4.67	0.68	12.90	87.10	8.90	1:2.23
Shorthorn[d]	6,155	3.94	3.32	4.99	0.70	12.81	87.19	8.87	1:2.26
Holstein	37,598	3.40	3.32	4.87	0.68	12.26	87.74	8.86	1:2.61

[a] C. W. Turner, Missouri Agr. Expt. Sta., *Bull.* **365** (1936).

[b] O. R. Overman, F. R. Sanmann, and K. E. Wright, Illinois Agr. Expt. Sta., *Bull.* **325** (1929).

[c] C. L. Roadhouse and J. L. Henderson, *The Market-Milk Industry.* McGraw-Hill, New York, 1941.

[d] C. H. Eccles, W. B. Combs, and H. Macy, *Milk and Milk Products.* McGraw-Hill, New York, 1936.

Most of the variations in the composition of milk disappear into an average because of the mixing of herd milk on a large scale. The breed of cow, however, has a very considerable effect.

The yield of milk is inversely proportional to the energy value, *i. e.*, the calorific value of the total solids per unit weight of milk. In other words, the greater the total volume of milk given by any one cow, the lower the total solid content.

Table 48 gives the average production of milk and butterfat of the cows having official yearly records in the breed associations.

TABLE 48

AVERAGE YEARLY PRODUCTION OF MILK AND BUTTERFAT OF THE COWS OF DIFFERENT BREEDS HAVING OFFICIAL YEARLY RECORDS TO JAN. 1, 1941[a]

Breeds	Advanced register or register of merit				Herd-improvement register			
	Records of cows and heifers	Milk, lbs.	Butterfat		Records of cows and heifers	Milk, lbs.	Butterfat	
			Quantity, lbs.	Test, %			Quantity, lbs.	Test, %
Ayrshire	7,129	10,469	416	4.0	30,593	8,488	343	4.0
Brown Swiss	1,195	13,669	552	4.0	984[b]	8,577	353	4.1
Guernsey	64,976	10,105	502	5.0	11,887	8,591	423	4.9
Holstein-Friesian	45,445	16,737	574	3 4	83,715[c]	11,208	385	3.4
Jersey	63,044[d]	8,584	460	5.4	43,978[e]	6,919	366	5.3

[a] A. B. Nystrom, U. S. Dept. Agr., *Farmers' Bull.* **1443**, rev. (1942).
[b] Up to Jan. 1, 1938.
[c] Up to Oct. 1, 1940.
[d] Includes 31,628 305-day records.
[e] Up to Jan. 1, 1940.

(*i*) *Fat, Protein, and Energy Relationship*[19]

The percentage of fat in milk may be taken as a rough measure of the protein content and energy value because the concentration of lactose, which contributes materially to the energy value, is relatively constant. While the fat and protein concentrations vary and may under some unusual conditions vary independently of each other, in general there is a definite relation between them. Haecker[20] determined the concentrations of fat, carbohydrate, and protein in 543 samples of milk. Armsby[21] calculated the total energy value of these milks taking 1 g. of fat as equivalent to 9.23 cal., 1 g. of protein as equivalent to 5.7 cal., and 1 g. of lactose as equivalent to 4.1 cal. The following equation represents these relationships:[22]

$$\%\ \text{protein} = 1.46 + 0.4\%\ \text{fat}$$

Overman and Sanmann[23] reported the fat, carbohydrate, protein, and energy content of 212 samples of milk, and Overman, Sanmann, and Wright[24] determined the fat, carbohydrate, and protein concentrations of 1999 samples. The relation of fat to protein in these milks were expressed by Mitchell[25] as

$$\%\ \text{protein} = 2.0 + 0.4\%\ \text{fat}$$

---

[19] P. A. Wright, E. F. Deysher, and C. A. Cary, U. S. Dept. Agr., *Yearbook, Separate No.* **1704** (1940).

[20] T. L. Haecker, Minnesota Agr. Expt. Sta., *Bull.* **140** (1914).

[21] H. P. Armsby, *Nutrition of Farm Animals.* Macmillan, New York, 1917.

[22] W. L. Gaines, *J. Dairy Sci.*, **8**, 486 (1925).

[23] O. R. Overman and F. P. Sanmann, Illinois Agr. Expt. Sta., *Bull.* **282** (1926).

[24] O. R. Overman, F. P. Sanmann and K. E. Wright, Illinois Agr. Expt. Sta., *Bull.* **325** (1929).

[25] H. H. Mitchell, Natl. Research Council, *Bull.* **67** (1929).

Overman and Gaines[26] concluded that the energy content of milk containing 4% of fat is approximately 341 cal. per lb. or 750 cal. per kilo. They suggested that with any breed of cows the following equation was applicable:

$$\text{weight of 4\% milk equivalent in energy value} = 0.4 \times \text{weight of milk yield} + 15 \times \text{\% fat}$$

Workers at the Ohio Agricultural Experiment Station concluded that "in samples of milk testing 2.78% of butterfat the percentages of protein and fat are approximately equal. Above this point the protein increases approximately 0.42 times as fast as the fat." The following formula expresses this relationship:

$$\text{\% protein} = 2.78 + 0.42\ (\text{\% fat},\ 2.78)$$

Table 49 summarizes these data. The results of the Ohio values are intermediate between the values calculated for the Haecker[27] milks and those analyzed by Overman, Sanmann, and Wright.[28]

TABLE 49

RELATION BETWEEN THE PROTEIN, FAT, AND ENERGY VALUES OF MILK[a]

Fat, %	Overman, Sanmann, and Wright[b]			Haecker			Ohio analyses, protein calculated %
	Protein, %		Energy calculated, cal./lb.[d]	Protein, %		Energy calculated, cal./lb.[e]	
	Found	Calculated[c]		Found	Calculated		
3.0	3.07	3.20	290	2.68	2.66	278	2.87
3.5	3.39	3.40	315	2.81	2.86	306	3.08
4.0	3.54	3.60	341	3.08	3.06	336	3.29
4.5	3.83	3.80	367	3.27	3.26	365	3.50
5.0	4.02	4.00	392	3.45	3.46	390	3.71
5.5	4.18	4.20	418	3.65	3.66	415	3.92
6.0	4.50	4.40	443	3.82	3.86	440	4.13
6.5	4.63[f]	4.60	469	4.02	4.06	467	4.34
7.0	4.91	4.80	494	4.22	4.26	492	4.55

[a] P. A. Wright, E. F. Deysher, and C. A. Cary, "Food and Life," U. S. Dept. Agr., *Yearbook* (1939), p. 639.

[b] Average for all breeds for which analyses are given.

[c] Calculated, using Mitchell's equation given in text.

[d] Calculated, using 341 cal. per lb. for 4% milk and equation in text for calculating the 4% milk equivalent in energy content to that yielded.

[e] Calculated by Armsby.

[f] Data for Jersey breed omitted.

(j) *Fat, Solids-Not-Fat, and Total Solids Relationship*

The variation in composition of milk because of changes in the solids-not-fat content is influenced by the factors previously mentioned (page 409). The solids-not-fat content of milk, however, changes at a slower rate than does the fat content. Based on average values, the solids-not-fat increase 0.4% for an increase of 1% in fat. This result was obtained by Jacobson[29] on analyses of 100,000 samples from milk plants.

---

[26] O. R. Overman and W. L. Gaines, *J. Agr. Research*, **46**, 1109 (1933).

[27] T. L. Haecker, Minnesota Agr. Expt. Sta., *Bull.* **140** (1914).

[28] O. R. Overman and F. P. Sanmann, Illinois Agr. Expt. Sta., *Bull.* **282** (1926).

[29] M. Jacobson, *J. Dairy Sci.*, **17**, 171 (1936).

The work performed by Overman, Sanmann, and Wright,[30] however, and that of the New York State Food Laboratory[31] do not substantiate such a linear relationship. The values of Overman and coworkers varied from a maximum of 0.61% increase in solids-not-fat content for a 1% increase in fat content, to a minimum of 0.25%, with an average value of 0.37%. These variations were largely attributable to breed differences. The work of the New York State Food Laboratory, which consisted of analyses of 2045 samples of herd milk, showed that there was a high degree of relationship between the fat and total solids content but that there was a low degree of relationship between the fat and solids-not-fat. The former relationship had a correlation coefficient value of 0.90, while the latter relationship had a correlation coefficient of 0.37. In other words, as the fat increased the total solids increased in linear proportion. This did not hold for the fat and solids-not-fat relationship. In incomplete milking or if there is retention of milk in the udder, these relationships do not hold because the solids-not-fat content is relatively constant throughout the milking process while the fat content increases toward the end of the milking.

TABLE 50

AVERAGE COMPOSITION OF MILKS OF MAMMALS[a]

Species	No. of samples	Water, %	Protein,[b] %	Fat, %	Lactose, %	Ash, %
Human	1154	87.43	1.63	3.75	6.98	0.21
Human	...	87.68	1.05	4.37	6.79	0.18
Cow	1998[c]	86.21	3.77	4.45	4.86	0.72
Cow	208[d]	87.90	3.13	3.65	4.50	0.72
Goat	326[e]	87.14	3.71	4.09	4.20	0.78
Ewe	2[e]	82.90	5.44	6.24	4.29	0.85
Egyptian buffalo	61	82.09	4.16	7.96	4.86	0.78
Chinese buffalo	30[e]	76.80	6.04	12.60	3.70	0.86
Philippine carabao	19[e]	78.46	5.88	10.35	4.32	0.84
Camel	...	87.61	2.98	5.38	3.26	0.70
Mare	104	89.04	2.69	1.59	6.14	0.51
Mare	.	90.23	2.30	0.78	6.42	0.44
Ass	...	89.70	2.10	1.50	6.40	0.30
Reindeer	...	63.30	10.30	22.46	2.50	1.44

[a] P. A. Wright, E. F. Deysher, and C. A. Cary, U. S. Dept. Agr., *Yearbook, Separate No.* **1704** (1940).
[b] 6.38 × nitrogen.
[c] 198 whole lactation periods from 14 Ayrshire, 16 Guernsey, 19 Holstein, and 15 Jersey pure bred cows and from 66 Guernsey-Holstein cross-bred cows.
[d] 208 New York herd samples.
[e] Number of animals used for samples.

(*k*) *Milk of Different Species*

The concentrations of the principal components of milk differ with various species of mammals. These values are given in Table 50. The chief characteristics of human milk are the high lactose content, the low protein content, and the low ash. The second set of values in Table 50 differ from the first set possibly because the milk was not sampled until the women had been in lactation for at least 2 months.[32]

---

[30] O. R. Overman, F. R. Sanmann, and K. E. Wright, Illinois Agr. Expt. Sta., *Bull.* **325** (1929).
[31] N. Y. State Food Laboratory, *Ann. Rept. N. Y. State Dept. Agr. Markets* (1933).

Table 51 indicates that the ratio of noncasein to casein protein in human milk is nearly four times the corresponding ratio in cow's milk, and that the ratio of nonprotein nitrogen to casein nitrogen in human milk is almost 13 times that in cow's milk.

TABLE 51

DISTRIBUTION OF NITROGEN IN HUMAN AND IN COW'S MILK[a]

Kind of milk	Total nitrogen, %	Casein nitrogen as percentage of total nitrogen, %	Noncasein protein nitrogen, as percentage of total nitrogen, %	Nonprotein nitrogen as percentage of total nitrogen, %
Human	0.1749	34.3	30.0	35.2
Cow's	0.497	76.1	18.0	5.9

[a] P. A. Wright, E. F. Deysher, and C. A. Cary, U. S. Dept. Agr., *Yearbook, Separate No.* **1704** (1940).

4. Grading of Milk

The United States Public Health Service Standard Milk Ordinance has the following standards for grading milk.

(*a*) *Grade A Raw Milk*

Grade A raw milk is raw milk the average bacterial plate count of which as determined under sections prescribed in the ordinance does not exceed 50,000 per cc., or the average direct microscopic count of which does not exceed 50,000 per cc. if clumps are counted or 200,000 per cc. if individual organisms are counted, or the average reduction time of which is not less than 8 hrs.: provided, that if it is to be pasteurized the corresponding limits shall be 200,000 per cc., 200,000 per cc., 800,000 per cc., and 6 hrs., respectively; and which is produced upon dairy farms conforming with all of the items of sanitation given in the regulation.

(*b*) *Grade B Raw Milk*

Grade B raw milk is raw milk which violates the bacterial standard and/or the abortion testing requirement for Grade A raw milk, but which conforms with all other requirements for Grade A raw milk, and has an average bacterial plate count not exceeding 1,000,000 per cc., or an average direct microscopic count not exceeding 1,000,000 per cc. if clumps are counted, or 4,000,000 per cc. if individual organisms are counted, or an average reduction time of not less than $3^1/_2$ hrs.

(*c*) *Grade C Raw Milk*

Grade C raw milk is raw milk which violates any of the requirements for Grade B raw milk.

(*d*) *Grade A Pasteurized Milk*

Grade A pasteurized milk is Grade A raw milk, with such exceptions as have been indicated if the milk is to be pasteurized, which has been pasteurized, cooled, and bottled in a milk plant conforming with all of the items of sanitation of the regulations and the average bacterial plate count of which at no time after pasteurization and until delivery exceeds 30,000 per cc.; it must be stoppered with a hooded cap; it must be kept at a temperature of less than 50° F. until delivered.

---

[32] P. A. Wright, E. F. Deysher, and C. A. Cary, U. S. Dept. Agr., *Yearbook, Separate No.* **1704** (1940).

(*e*) *Grade B Pasteurized Milk*

Grade B pasteurized milk is pasteurized milk which violates the bacterial standard for Grade A pasteurized milk and/or the provision of lip-cover caps or the requirement that Grade A raw milk be used, but which conforms with all other requirements for Grade A pasteurized milk, has been made from raw milk of not less than Grade B quality, and has an average bacterial plate count after pasteurization and before delivery not exceeding 50,000 per cc.

(*f*) *Grade C Pasteurized Milk*

Grade C pasteurized milk is pasteurized milk which violates any of the requirements for Grade B pasteurized milk.

5. Pasteurized Milk

The sale of raw or improperly pasteurized milk is prohibited in many cities and towns because such milk may constitute a health hazard. Pasteurized milk is defined by the U. S. Public Health Service as milk every particle of which has been subjected to a temperature not lower than 143° F. for not less than 30 min. or to at least 160° F. and holding at that temperature for at least 15 sec. The milk should then be promptly cooled to 50° F. or lower. In England and other countries milk must be held for half an hour at 145 to 150° F. for 30 min. to be properly pasteurized, followed by immediate cooling to 55° F. Other methods of pasteurization such as flash pasteurization, high-temperature pasteurization, high-temperature short-time pasteurization, stannanization, retarder-type low-temperature pasteurization and holder-type pasteurization have been described[38] and advocated. This subject is discussed in Volume II, Chapter XX.

In general, pasteurization has little effect on the flavor of milk and no significant effect on its nutritive value. There is some diminution of the vitamin C, vitamin B_1, and iodine content. On the other hand, pasteurized milk will keep considerably longer than raw milk and its digestibility is improved for infants. Most of the enzymes which milk contains are inactivated by the pasteurization process.

6. Certified Milk

Milk which is produced under exceptionally rigid conditions of cleanliness and sanitation so that its original bacteria count is very low has been marketed under the name of certified milk. Certified milk-raw is milk which conforms with the requirements of the American Association of Medical Milk Commissions. Certified milk-pasteurized is certified milk-raw which has been pasteurized, cooled, and bottled in a milk plant conforming with the requirements for Grade A pasteurized milk (see page 413). Formerly, the Sanitary Code of the City of New York required that certified milk-raw contain not more than 10,000 bacteria per cc. while certified milk-pasteurized contain not more than 500 bacteria per cc.

---

[38] G. S. Wilson, *Pasteurization of Milk.* Arnold, London, 1942.

7. Vitamin D Milk

Vitamin D milk is milk the vitamin D content of which has been increased by a method and in an amount approved by public health authorities. It was first introduced in 1932, primarily because ordinary market milk does not contain sufficient vitamin D to be a completely protective food as far as this vitamin is concerned. In 1943, about 310,000 qts. of vitamin D milk were sold per day in New York City. This represented about 10% of the total volume of household fluid milk sold daily in that city.

(*a*) *Irradiated Milk*

There are a number of different types of vitamin D milk marketed. One type, namely, irradiated milk, is produced by the direct irradiation of milk with ultraviolet light. Since milk is known to contain cholesterol, irradiated milk contains the cholesterol type of vitamin D.

(*b*) *Metabolized Vitamin D Milk*

Another type of vitamin D milk is known as metabolized vitamin D milk. It is produced by feeding the cow irradiated yeast which is known to contain ergosterol. In view of the basic difference in types of vitamin D in the milk one should expect a difference in potency of these milks when tested on rats and on chickens. There is little practical difference between the two milks, unit for unit, in respect to their effectiveness for rachitic infants.

(*c*) *Fortified Vitamin D Milk*

Another type of vitamin D milk is fortified vitamin D milk. It is made by adding to milk a vitamin D concentrate from cod-liver oil. The potency of fortified milk is adjusted by producers at approximately 400 U. S. P. Units per quart and it contains the cholesterol type of vitamin D.

The term fortified vitamin D milk has been extended to cover the use of other concentrates such as other fish-liver oils or irradiated ergosterol. The vitamin D concentrate is emulsified in an oil such as cottonseed oil or in cream and is mixed with the milk to be fortified (see Vol. II, Chapter XX).

8. Vitamin Fortified Milks

Some milk is prepared on a commercial scale which has been fortified with vitamins other than vitamin D. These are known as multivitamin milks. They are generally sold under trade names and are enriched with an adult's requirement of some of the vitamins. Vitamins A, B_1, B_2, C, nicotinic acid amide, calcium pantothenate, and vitamin D are used

9. Homogenized Milk

Homogenized milk is the milk product that has been mechanically treated in such a manner as to alter its physical properties, with particular reference to the condition and appearance of the fat globules. The U. S. Public Health Service defines homogenized milk as milk which has been treated in such manner as to ensure breakup of the fat globules to such an extent that after 48 hrs.' storage no visible cream separation occurs on the milk, and the fat percentage of the top 100 cc. of milk in a quart bottle, or of proportionate volumes in containers of other sizes, does not differ by more than 5% of itself from the fat percentage of the remaining milk as determined after thorough mixing.

Homogenization is a mechanical process by which particles of matter are reduced in size and intimately mixed during that process. When milk is homogenized, the fat particles are broken up to such an extent that they no longer form a cream layer. To do this, the milk is forced under high pressure which varies from 2000 to 4000 lbs. per sq. in., according to the machine, through a very small aperture, orifice, or valve, which is capable of being minutely adjusted to a definite size. In this process, the milk fat globules are reduced in size to the diameter of the orifice. The enormous increase in surface area that accompanies this reduction in size stabilizes the milk emulsion and prevents the settling of the cream. It has been shown that a pressure of 2500 lbs. gives almost the maximum reduction possible in curd tension.[34]

An alternative method of size reduction is to pass the milk through closely approximated vibrating surfaces such as disks or plates. The result obtained is about the same in both types of homogenizing machines. Sometimes homogenization is also termed viscolization.

This mechanical process is finding increasing use in the field of food processing. It is used in the manufacture of ice cream, cultured buttermilk, cultured sour cream, whipped cream, cream cheese, process or pasteurized cheese, mayonnaise and salad dressings, pie fillings, cream fillings, and in an ever-increasing number of food products.

One of the principal reasons adduced for the sale of homogenized milk is that such milk may be served from mechanical dispensers and still retain a uniform composition. Other advantages attributed to this type of milk are that it is more easily digested, probably because the curd tension has been reduced, and that it retards or inhibits the development of copper-induced oxidized flavors in milk. On the other hand, sunlight-induced oxidized flavors are produced more readily in homogenized milk. Homogenized milk does not differ significantly from unhomogenized milk in the development of acid, as shown by titratable acidity and *p*H estimations.[34]

10. Soft Curd Milk

Until relatively recent times it seldom occurred to anyone that cow's milk might be unsuitable for some human beings, particularly infants. The milk of cows produces, according to some investigators, a tough, leathery, tenacious mass when acted upon by gastric juice. Human milk yields a fine, flocculent curd under the same conditions. Because of these differences in curd structure, it is believed by some authorities that the former is more indigestible than the latter.

---

[34] C. J. Babcock, U. S. Dept. Agr., *Tech. Bull.* **832** (1942).

There are a number of methods available for the preparation of soft curd milk. One of these methods, namely, that of homogenization, has been discussed on page 416. Another method is to heat milk considerably above the temperature used for pasteurization. Such treatment produces a soft curd milk. Heat treatment probably accounts for the soft curd produced with evaporated milk or boiled normal milk.

A third method is to gather a herd of cows each one of which has the individual characteristic of producing milk of low curd tension. Since this characteristic property generally remains constant such herds can be assembled so that the herd milk produced has a curd tension of less than 30 g.

The base-exchange treatment of milk is another method for the production of soft curd milk. By this process, in which milk acidified with citric acid is passed through a zeolite filter, about 20% of the calcium is removed with a slight alteration in the phosphorus content and salt content of the milk.

Milk of low curd tension may be obtained by the addition of the sodium salts of hexametaphosphate, pyrophosphate, metaphosphate, and citrate. Another method is to treat milk with a proteolytic enzyme such as trypsin. Still another method is to subject milk to intense high-frequency vibration in an oscillating device. No pressure is used. This type is known as sonic soft curd milk.

One cannot state categorically that soft curd milk produced by the addition of salt, the base-exchange method, or other treatment does not have some place in our diet. This place should be limited, however, until it is proved that such a really important change is needed, and until it can be demonstrated that the price differential between this type of milk and normal milk is negligible.

11. Reconstituted Milk

Reconstituted, reconstructed, or recombined milk is a product resulting from the recombining of milk components with water, and which complies with the standards of milk fat and solids-not-fat of normal milk. Remade, reconstituted, or reconstructed milk is milk that has been made from milk powder either skimmed or whole, from cream, from evaporated or frozen milk, or from any combination of these products and butter or butter oil. In general, it is any type of milk manufactured by any process other than the normal production by cows.

In order to produce a properly reconstructed milk it is generally homogenized. Such milk is generally made according to a formula and if the milk is to comply with the standards of normal milk, each ingredient must be fully incorporated. Often the sole ingredients are butter or butter oil, skim milk powder, and water. In order for such a mixture to remain in suspension, it must be properly homogenized, otherwise a sediment will settle out.

12. Frozen Milk

Investigations have shown that fluid milk can be concentrated to one-third of its volume, sealed in containers, frozen without agitation at a temperature of about 0° F., held for about 5 weeks and then thawed and reconstituted to fluid consistency without noticeably impairing the quality of the product for use as fluid milk.

It has been suggested that this process may be used as a means of furnishing milk to territories which are without an adequate or satisfactory supply of fresh fluid milk. Such a product would have advantages over other storage products such as sweetened condensed milk or evaporated milk.

When reconstituted, concentrated frozen milk yields a product which generally has a slight tallowy flavor and a marked reduction in curd tension. Very likely the protein undergoes some marked changes on storage under those conditions and when thawed does not have the same properties it had prior to freezing.

The length of time frozen milk can be held, and then properly reconstructed, is limited, although 12 weeks seem possible, and since this type of milk must be held under refrigeration, the cost of holding is great enough to prevent its competition with normal milk.

II. CREAM

Cream or sweet cream is the portion of milk, rich in butterfat, that is separated from the remainder of the milk by means of centrifugal force, or that rises to the surface of milk on standing. By far most of the cream sold is the product obtained by means of centrifugal separators. The most important component of cream is, therefore, butterfat.

The different States, cities, and communities of the United States have different standards for grades of cream. According to Food and Drug Administration definitions, cream is the sweet fatty liquid or semiliquid separated from cow's milk. Sweet whole milk or sweet skim milk may be added to it, but cream must contain at least 18% butterfat.

Light cream, table cream, or coffee cream, under these definitions, must contain at least 18% and not more than 30% butterfat. This cream may be homogenized. Homogenized light cream does not whip easily. Light whipping cream must contain not less than 30% butterfat and may contain up to 36%. This type of cream is usually not homogenized. Heavy whipping cream must contain at least 36% of butterfat.

The minimum requirements for fat as given in the Sanitary Code of the City of New York are 18% for light cream, 23% for medium cream, and 36% for heavy cream. The exigencies of the second World War caused the Office of Price Administration to eliminate all grades above that of light cream.

The factors which influence the amount of fat in cream are: (1) the fat content of the milk, (2) the position of the cream outlet, (3) the rate of inflow of milk, (4) the speed of the centrifuge, (5) the temperature of the milk, and (6) the quantity of water or skim milk used to flush the separator bowl.

The greater the fat content of the milk, the greater will be the fat content of the resultant cream. The factors influencing the fat content of milk have been discussed on page 409. A centrifugal separator has two principal outlets, one for cream near the center of the bowl and the other for skim milk at its periphery. The farther the position of the cream outlet from the center of the bowl, the less the fat content of the resultant cream. Separators with adjustable skim milk outlets which depend on the size of the opening, provide richer or lower fat cream by an adjustment of the outlet. The smaller the skim milk outlet, the larger the cream output and consequently the lower the fat content of the cream.

TABLE 52

COMPOSITION OF CREAM

Type	Fat, %	Solids-not-fat, %	Total solids, %
	16.02	7.43	23.45
	17.01	7.34	24.35
Light	18.00	7.25	25.25
	19.04	7.16	26.20
	20.03	7.07	27.10
	21.02	6.98	28.05
	22.00	6.90	28.90
Medium	23.00	6.81	29.81
	24.00	6.72	30.72
	25.00	6.63	31.63
	26.00	6.54	32.54
	27.00	6.46	33.46
	28.00	6.37	34.37
	29.00	6.28	35.28
Whipping	30.00	6.19	36.19
	31.00	6.10	37.10
	32.00	6.09	38.09
	33.00	5.92	38.92
	34.00	5.83	39.83
	35.00	5.75	40.75
Heavy	36.00	5.66	41.66
	37.00	5.57	42.57
	38.00	5.48	43.48
	39.00	5.39	44.39
	40.00	5.30	45.30
	45.00	4.86	49.86
	50.00	4.42	54.42
	55.00	3.98	53.98

Since the discharge of skim milk from a skim milk outlet of a given aperture is relatively constant, for a given speed, increasing the inflow of milk causes the milk in excess of that which can escape through the skim milk outlet to pass out of the cream outlet. This action will tend to decrease the fat content of the resultant cream. On the other hand, increase of speed increases the centrifugal force with a consequent increase in flow through the skim milk outlet. Thus increase in speed of the separator increases the fat content of the cream.

The warmer the milk, the less the fat content of the cream. This is due to the increase in discharge through the cream outlet.

In order to obtain the cream remaining in the bowl after separation, it is flushed out with water or skim milk. If this is done so that just sufficient water or skim milk necessary to rinse the bowl is used, if the water and skim milk are run in slowly and a large amount of milk is used in the separation, the fat content of the cream may not be materially affected. But if small amounts of milk are separated and care is not taken in the separation then a considerable decrease in fat content may result.

A typical light cream contains 20% fat, 2.9% protein, 4.0% lactose, 0.6% ash, 27.5% total solids, and 72.5% water. A heavy cream contains about 36% fat, 2.3% protein, 3.2% lactose, 0.5% ash, 42% total solids, and 58% water.

Assuming that the percentage of fat in cream is related to the percentage of total solids according to the following formula

$$\%\ \text{fat} = 1.097 \times \%\ \text{total solids} - 9\ 70$$

which formula is based on the assumption that for every 100 parts of water there are 9.7 parts of milk solids-not-fat,[35] we can calculate the approximate composition of cream as shown in Table 52.

The quality of cream as judged by the layman depends, in a large measure, upon its viscosity or thickness. Thus the more viscous the cream appears, the better or richer it appears to the consumer. This factor of viscosity is even of greater importance in such products as sour cream and buttermilk. By high temperature pasteurization, inoculation with a proper starter, subsequent homogenization with incorporation of air, and, finally, aging, sour cream and buttermilk of almost any desired viscosity can be obtained. Even in sweet cream, a definite thickening or gelatinizing effect can be obtained merely by pasteurization and homogenization.

1. Sour Cream

Sour cream is defined as cream the acidity of which is more than 0.20%, expressed as lactic acid. It is a product which is sometimes called cultured cream. It is in much favor with people of Russian, Jewish, Polish, and German extraction. Formerly sour cream was prepared by permitting sweet cream to sour naturally.

Most of the commercial product is produced by inoculation of the cream with a bacterial culture. The product prepared under rigid control yields a very thick plastic mass.

The native American prejudice against sour cream is unfounded. If the taste is acquired, it becomes a favorite with many. It is useful in salads, with fruits and vegetables, and with borsch, a soup made with beets.

One of the principal sources of cream used in the modern manufacture of sour cream is storage cream. Storage cream is sweet cream surplus, which is generally gathered during the overflow months of May and June. It is centrifugally separated but not to so great an extent as plastic cream, so that it has a fat content of about 50%. This product is then frozen solid.

---

[35] T. Mojonnier and H. C. Troy, *Technical Control of Dairy Products*. Mojonnier Bros., Chicago, 1925.

The material is thawed and mixed with sufficient sweet milk, or with skim milk powder and water, to reduce the fat content to 18%. Then it is homogenized, during which process sufficient air is incorporated into the material to yield about a 10% overrun. After homogenization, it is pasteurized at 143–170° F. depending on the process used, for 30 min. It is cooled to room temperature and placed into 40-qt. cans. The homogenized and pasteurized cream is then inoculated with a lactic bacteria starter and allowed to stand for 24–48 hrs. at room temperature, the length of time depending upon the amount of starter added, the thickness of the cream desired, and the demand of the consuming public at the moment.

2. Plastic Cream

Plastic cream is chilled cream of 65–83% butterfat content, generally, however, covering the range of 80–83% butterfat. It is true cream for, although its butterfat content is similar in quantity to that of butter, the fat of plastic cream is dispersed throughout its water, in contradistinction to butter, in which the water is dispersed in the fat. In other words, plastic cream, like milk or cream, is an oil-in-water emulsion, whereas butter is a water-in-oil emulsion.

Plastic cream is made by an intense centrifugal treatment which skims out nine-tenths of the serum and serum solids. Fluid cream of any fat content may be turned into plastic cream by heating the cream and passing it through a special centrifugal separator. It may then be pasteurized. The process may be used in the salvage of poor and dirty cream, for the extreme method of separation removes the insoluble filth as a residue of bowl-cake sediment.

Plastic cream may be distinguished from butter in that it is crumbly in texture whereas butter is greasy. It is shipped in cartons and tubs, like butter, as it is not fluid. One of its chief uses is the manufacture of a type of cream cheese. This product is also recommended for the manufacture of reconstituted cream and whipping cream.

3. Whipped Cream

Light cream that is neither pasteurized nor aged will not whip with ease. Formerly whipping cream was defined as cream which contains not less than 30% of milk fat. After aging, light cream will whip, although using a cream of higher fat content will yield better results. By the aging process the acidity is slightly increased. The acid subsequently affects the proteins, namely, casein and lactalbumin, giving them the ability to form the gelatinous consistency which is necessary to entrap the air while the cream is being whipped. This may be arranged more quickly by the addition of 0.1 to 0.3% of commercial lactic acid or by the addition of calcium sucrate or sucrate of lime (Viscogen). The addition of these substances is prohibited by most communities.

Another method for the manufacture of whipped cream is that of impregnation with nitrous oxide. Other gases have been used but it has been found that none of these can give the high overrun or swell in volume that is obtained with nitrous oxide. This type of whipped cream is distributed at times in cylinders under pressure, or it may be made at the dispensing counter with the aid of cylinders of nitrous oxide or by use of siphoning apparatus containing bulbs of the gas. By the use of this gas an overrun or increase in volume of as much as 450% can be obtained. With the siphon arrangement single portions of whipped cream can be served.

Products resembling whipped cream are sometimes made by whipping, *i. e.*, agitating with the incorporation of air, mixtures of milk powder with some stabilizing agent such as acacia, tragacanth, or other type of gum or gelatin.

III. BUTTER

When cream is churned the fat droplets coalesce and form progressively larger clusters of fat globules. These grains finally break away from the surrounding liquid and form a semisolid or plastic material called butter. It is made exclusively from milk or cream, or both with or without the addition of common salt and with or without the addition of coloring matter. It must contain not less than 80% butterfat.

Butter is generally sold as salt or sweet butter and in the form of tub, cube, print, or whipped butter.

Either sweet or sour cream may be used for the manufacture of butter. The cream is "neutralized," that is, its acidity is adjusted by the addition of calcium hydroxide, magnesium hydroxide, calcium carbonate, sodium carbonate, sodium bicarbonate, etc. After pasteurization, starters are added so that the cream can be ripened. The cream is then churned in which process the colloidal system is inverted from the oil-in-water system of cream to the water-in-oil system of butter. Washing, salting, and working the butter mass prepares it for the market.

Whipped butter is a more recent departure in butter making. It is the product of the process of incorporating air into the butter mass by means of mechanical agitation or whipping. This produces a product which will spread as easily in the winter as in the summer. It also gives the butter a light yellowish color, a fluffy appearance, and a creamy taste. Since butter is generally sold by weight, the incorporation of air makes little difference in its sale in contradistinction to ice cream which is sold by volume and in which the incorporation of air makes a large difference. In the whipping of butter, the size of the butterfat particles is reduced. Thus in unwhipped butter the size of the particles range from 6–7 to 15–20 μ, while in whipped butter the particle-size range is 1–2 to 6–7 μ.

1. Composition of Butter

The chief component of butter is, of course, butterfat, which comprises at least 80% of the food material. Thus the average composition of 672 samples[26] was

---

[26] North Dakota State Laboratories Dept., *Bull.* 67 (1942).

80.9% fat, 16.1% moisture, and 2.3% salt. Table 53 gives some representative values of butter compositions, while Table 54 gives the percentage of the component fatty acids of butterfat.

TABLE 53

COMPOSITION OF CREAMERY BUTTER

	No. of samples	Fat, %	Protein (curd), %	Lactose, %	Salt, %	Water, %
Maximum	695[a]	86.91	3.42	..	5.26	16.83
Minimum	..	77.64	0.20	..	0.92	10.52
Average	..	82.41	1.18	..	2.51	13.90
Average	573[b]	81.12	0.98	..	2.44	15.46
Average[c]	..	81.0	0.6	0.4	2.5	15.5
Whipped sweet	..	80.1	..	..	0.7[d]	19.2

[a] M. G. Thomburg, *Chicago Daily Produce*, No. **10**, 14 (1925).

[b] S. C. Thompson, R. H. Shaw, and R. P. Norton, U. S. Dept. Agr., Bur. Animal Industry, *Bull.* **149** (1912).

[c] C. Chatfield and G. Adams, U. S. Dept. Agr., *Circ.* **549** (1940).

[d] Curd and salt.

TABLE 54

COMPONENT FATTY ACIDS OF BUTTERFAT, %

Fatty acid	a	b
Butyric	2.6– 3.5	2.4
Caproic	1.3– 1.9	1.1
Caprylic	0.7– 1.6	0.7
Capric	1.8– 3.6	3.6
Lauric	3.2– 5.7	2.3
Myristic	6.9–11.1	12.6
Palmitic	22.8–29.1	28.5
Stearic	6.5–12.5	12.1[c]
Arachidic (?)	0.6– 0.9	..
Oleic	31.3–41.3	30.5
Linoleic	3.6– 5.1	..
Decenoic	...	0.2
Tetradecenoic	...	1.1
Hexadecenoic	...	3.3
Arachidonic	...	1.6

[a] T. P. Hilditch, *Analyst*, **62**, 250 (1937).

[b] J. A. Gamble, N. R. Ellis, and A. K. Besley, U. S. Dept. Agr., *Tech. Bull.* **671** (1939).

[c] Includes small quantities of higher saturated acids.

2. Quality Standardization of Butter

The first work on quality standardization attempted by the Department of Agriculture for dairy products had in view the establishment of a uniform basis for the grading of butter and cheese. This work was begun in 1918. The standards subsequently developed by the Dept. of Agriculture followed closely the established trade practice, but the grades were made much more definite and were applied uniformly in all markets by the government graders. These official standards were promulgated under the provisions of the farm products inspection law.

For the purposes of grading, butter is classified into six classes: (1) dairy, (2) creamery, (3) packing-stock, (4) ladled, (5) process or renovated, (6) grease. Dairy butter implies a butter made on a farm. Creamery butter implies butter made in a creamery or factory. Process butter, *i. e.*, renovated or reconstructed butter, is made from packing-stock butter, the trade name for miscellaneous lots of butter collected, pressed into one container, and offered for resale. This packing-stock butter is melted, refined, and churned into an almost new product. The molten butterfat is sometimes filtered to free it of filth and dirt. Then water, salt, and some cream are added and the entire material is rechurned in order to make it resemble the natural product. Grease butter is the lowest grade and is considered unfit for food.

In scoring the first three classes of butter, maximum ratings are given to various factors as follows: flavor, 45; body, 25; color, 15; salt, 10; package, 5; total, 100. Butter scoring above 93 or 94 must be sweet, fresh, mild, and clean in flavor. Diminishing desirability is expressed by lowered scores. In actual official practice, the rating of each quality factor is somewhat modified to give proper recognition to actual market requirements. Any butter scoring below 75 is classified as grease butter and as such is considered unfit for food.

Regardless of the score given to butter on the above scale of rating, any butter falling below the 80% minimum for butterfat content or any butter which is filthy or decomposed is an adulterated product.

Only butter officially scoring 92 or more could formerly be accompanied by a dated "certificate of quality" issued by authority of the U. S. Dept. of Agriculture. In 1943, the Food Distribution Administration, U. S. Dept. of Agriculture, issued revised grade standards. Provision was made for the elevation of top grade, 91 Score butter to the 92 Score level. Straight or short 91's were scored 90. Letters were substituted for the numerical designations, as follows: AA for U. S. 93 Score; A for U. S. 92 Score; B for U. S. 90 Score; C for U. S. 89 Score; and CG or cooking grade for butter below U. S. 89 Score.

3. Butter Oil and Ghee

Butter oil is the product made by clarifying butter by means of melting and centrifuging. It contains practically no water or any of the other components of butter, and, consequently, is almost entirely butterfat.

Ghee is a very similar product. The butter made from buffalo milk is clarified by boiling and filtration so that a product resembling an oil is obtained. This product will not turn rancid as easily as butter.

IV. EVAPORATED MILK

The production of concentrated milk on a commercial scale started at about the same time that factory production of butter and cheese began. Thus Gail Borden was granted a patent for the manufacture of milk in which a portion of the water was removed in 1856. Evaporated milk is the product resulting from the evaporation of a considerable portion of the water from milk, or from milk with the adjustment, if necessary, of the ratio of fat to nonfat solids by the addition or abstraction of cream. Maximum, minimum, and average compositions of this product are given in Table 55.

Evaporated milk is defined by the Food and Drug Administration as sweet whole cow's milk evaporated so that it contains not less than 7.9% by weight of milk fat and 25.9% of total milk solids. The evaporated milk must be sealed in a container and processed by heat so that it will not spoil. To ensure smoothness, disodium phosphate or sodium citrate, or both, or calcium chloride may be added but not more than 0.1% of these stabilizers may be contained in the final evaporated milk product.

Evaporated milk may also be reinforced with vitamin D. The enrichment may be made by the irradiation process or by the addition of an edible oil which contains the vitamin. The amount of oil added, however, must not weigh more than 0.01% of the total weight of the final product. The amount of vitamin D added must be not less than 7.5 U. S. P. Units per oz., and the container must be labeled to indicate the addition of the vitamin.

TABLE 55

COMPOSITION OF EVAPORATED MILK

	Fat, %	Protein, %	Lactose, %	Ash, %	Total solids, %	Water, %
Maximum[a, b]	8.90	7.08	10.84	1.75	27.40	74.57
Minimum[a, b]	7.90	6.38	9.38	1.34	25.43	72.60
Average[a, b]	8.22	6.71	10.13	1.55	26.37	73.63
Maximum[c]	8.1	...	...	...	27.7	74.4
Minimum[c]	7.9	...	...	...	25.6	72.3
Average[c, d]	8.02	...	...	...	26.5	73.5
Maximum[e]	8.0	7.2	..	1.7	27.6	74.6
Minimum[e]	7.8	6.3	...	1.5	25.4	72.4
Average[e, f]	7.84	6.8	...	1.6	26.1	73.9

[a] J. P. Street, Connecticut Agr. Expt. Sta., *Bull.* 213 (1919).

[b] L. A. Rogers, *Fundamentals of Dairy Science.* Chemical Catalog Co., New York, 1928.

[c] North Dakota State Laboratories Dept., *Bull.* 67 (1942).

[d] 12 commercial samples.

[e] North Dakota State Regulatory Dept., *Bull.* 47 (1935).

[f] 19 commercial samples.

V. CONCENTRATED MILK

Concentrated milk or plain condensed milk should not be confused with sweetened condensed milk sold in cans to consumers. It is whole milk from which part of the water is removed by evaporation in a vacuum pan. It is generally sold in bulk to bakeries and other establishments which use milk in large quantities as an ingredient in their manufactured products.

Under an official standard of identity, the Food and Drug Administration requires concentrated milk to conform to the definition of evaporated milk except that it need not be sterilized nor need it be packed in hermetically sealed containers.

VI. CONDENSED MILK

Condensed milks may actually be divided into two large groups of unsweetened condensed and sweetened condensed milk. The former is generally designated as evaporated milk which has been discussed in a previous paragraph. Condensed milk is generally taken to mean the sweetened product and if an unsweetened condensed milk is sold in bulk it is designated as plain condensed milk or concentrated milk.

Thus evaporated milk and condensed milk are two different products from both composition and method of production.

Condensed milk, sweetened condensed milk is the product resulting from the evaporation of a considerable portion of the water from milk to which sugar or dextrose or both have been added. The composition of sweetened condensed milk, as given by a typical analysis, is: fat, 8.5%; protein, 8.1%; lactose and sugar, 54.7%; ash, 1.7%; water, 27.0%.

Sweetened condensed milk is defined as the liquid or semiliquid food made by evaporating a mixture of sweet milk and refined sugar (sucrose) or any combination of refined sugar (sucrose) and refined corn sugar (dextrose) to such a point that the finished sweetened product contains not less than 28% of total milk solids and not less than 8.5% of milk fat. The quantity of sucrose or combination of sucrose and dextrose should be sufficient to prevent spoilage.

VII. ICE CREAM

Ice cream is the frozen product made from a combination of milk products and 2 or more of the following ingredients: eggs, water, and sugar with harmless flavoring and harmless coloring matter, with or without stabilizer, and in the manufacture of which freezing may be accompanied by agitation of the ingredients. The milk products generally used are one or more of the following: cream, butter, butter oil, milk, evaporated milk, skimmed milk, condensed milk, sweetened condensed milk, condensed skimmed milk, sweetened condensed skimmed milk, dried milk, dried skimmed milk. Ice cream was formerly considered more nearly a confection than a staple article of our diet, but for many people, especially children, it is a regular item of their daily fare. Consequently its manufacture and sale should be as rigidly controlled as that of milk.

1. Composition of Ice Cream

In Table 56 are tabulated the ingredients used to prepare ice cream mixes for ice cream containing 10% of butterfat. Thus it may be seen from this table that the composition of any ice cream depends upon the materials used in its preparation. Since ice cream is most often prepared by freezing while agitating, large amounts of air are incorporated into the product. Thus air is one of the components of ice cream. The increase in volume which results from this agitation during the freezing process is known as *overrun*.

2. Standards for Ice Cream

Standards for ice cream vary considerably from community to community. Almost all have different fat standards for plain or vanilla ice cream as compared with fruit, nut, chocolate, or similarly flavored ice cream. These codes probably assume that a certain amount of food value has been added because of the incorporation of fruits, nuts, and cocoa which compensates for the lower fat standard. Often, however, only artificial flavors or colors are used and no compensating food is added.

Ice cream is often sold with type names. *Bisque* is usually plain ice cream of high fat content, from 16 to 20%, with added cake confections such as macaroons, or sponge cake. *Aufait* is a type of brick ice cream. *Parfait* is ice cream rich in fat, from 16 to 20%, with or without fruit or nuts and with eggs. *Pudding* is ice cream of high fat content containing eggs and nuts, or the like. *Custard* is the type of ice cream made with eggs which is generally frozen to a plastic rather than a solid state. Frozen custard is synonymous with custard ice cream. French ice cream contains a high fat content and also sufficient eggs to give the product a pronounced yellow color. It also generally contains particles of vanilla bean, particularly when sold as French vanilla.

TABLE 56

INGREDIENTS OF ICE CREAM MIXES FOR 10% ICE CREAM ON 1000-LB. BASIS[a,b]

18% cream	366.1	415.6	210.7[c]	491.5	385.5	500.8
Evaporated milk	379.7	....	....	....	....	....
Whole milk	109.2	220.4	416.7	....	311.5	259.2
Plain condensed whole milk	....	219.0	....	....	....	....
Condensed skim milk	....	....	227.6	....	....	....
Sweet condensed whole milk	....	....	....	....	238.0	....
Sweet condensed skim milk	....	....	....	285.0	....	163.9
Sugar	140.0	140.0	140.0	10.3	40.0	71.1
Gelatin	5.0	5.0	5.0	5.0	5.0	5.0
Water	....	....	....	208.2	....	....
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
Skim milk powder	45.8	110.0	....	....	....	....
Butter	89.6	119.2	68.9	117.8	....	....
Whole milk	719.6	....	1224.0	....	....	....
Skim milk	....	....	...	1174.0	....	....
Sugar	140.0	140.0	140.0	140.0	....	....
Gelatin	5.0	5.0	5.7	5.7	....	....
Water	....	625.8	....	....	....	....
Total	1000.0	1000.0	1438.6	1437.5	....	....
Water to be removed by vacuum pan	....	...	438.6	437.5	....	....

[a] M. B. Jacobs, *Chemical Analysis of Foods and Food Products.* Van Nostrand, New York, 1938.

[b] T. Mojonnier and H. C. Troy, *Technical Control of Dairy Products.* Mojonnier Bros., Chicago, 1925.

[c] 40% cream.

VIII. MILK SHERBETS AND ICES

In some communities, products are permitted to be sold which are allied to ice cream in that they are frozen desserts. These products are often called milk sherbets and ices or ice sherbets. In New York State, a milk sherbet must not contain more than a total of 5% milk solids. Thus sherbets can be made with most of the ingredients mentioned for ice cream.

Ices or ice sherbets are frozen desserts that do not contain any milk solids. They are generally made from sugar, water, artificial flavoring and coloring matters, and some stabilizer.

IX. CHEESE

Cheese is the food product made from the separated curd obtained by coagulating the casein of milk, skimmed milk, or milk enriched with cream. The coagulation is accomplished by means of rennet or other suitable enzyme, lactic fermentation, or by a combination of the two. The curd may be modified by heat, pressure, ripening ferments, special molds, or suitable seasoning. Certain varieties, such as Roquefort cheese, for example, which is made from the milk of sheep, are made from the milk of animals other than the cow. Cheese unqualified generally means American Cheddar cheese. There are hundreds of names for cheese, native and foreign, although there are only about 18 varieties. These may all be classified under the headings: whole milk cheese, part skim milk cheese, skim milk cheese, pasteurized cheese and process cheese, or as hard or soft curd cheese. Hard and soft curd cheese may be further subdivided as follows:[37]

Hard	Very hard	Without gas holes	Cheddar
		With gas holes	Swiss
	Semihard	Ripened by molds	Roquefort
		Ripened by bacteria	Brick
Soft	Ripened by bacteria		Limburger
	Ripened by mold		Camembert
	Unripened		Cottage

TABLE 57

COMPOSITION OF VARIETIES OF CHEESE[a, b]

Variety	Water, %	Fat, %	Protein, %	Ash, %
Brick	42.5	30.7	21.1	3.0
Caciocavallo	35.0	22.0	34.3	7.0
Camembert	47.9	26.3	22.2	4.1
Cheddar	36.8	33.8	23.7	5.6
Cottage	69.8	1.0	23.3	1.9
Cream	42.7	39.9	14.5	1.9
Edam	38.1	22.7	30.9	6.2
Emmenthaler	33.0	30.5	30.4	4.2
Gorgonzola	37.3	34.7	25.2	3.8
Gouda	38.1	24.5	29.6	6.1
Gruyére	30.0	28.2	33.0	4.0
Limburger	54.8	19.6	21.3	5.2
Munster	40.6	31.0	22.2	4.6
Neufchâtel	52.1	23.5	19.3	5.0
Parmesan	17.0	22.7	49.4	7.6
Pecorino	29.8	30.5	33.5	6.2
Romano	29.6	27.7	31.2	8.7
Roquefort	38.7	32.2	21.4	6.1
Sapsago	47.8	2.0	41.6	11.9
Stilton	33.6	31.2	29.0	3.0
Swiss	33.9	30.6	29.2	4.2

No typical analyses are given for process cheese and for filled cheese. These should follow closely the varietal name.

[a] C. F. Doane and H. W. Lawson, revised by K. J. Matheson, U. S. Dept. Agr., *Bull.* 608 (1932).

[b] M. B. Jacobs, *Chemical Analysis of Foods and Food Products.* Van Nostrand, New York, 1938.

---

[37] C. H. Eckles, W. B. Combs, and H. Macy, *Milk and Milk Products.* McGraw-Hill, New York, 1936.

The composition of some of the more well-known varieties of cheese are given in Table 57.

The classifications other than process cheese and pasteurized cheese are self-explanatory. The entire process of making cheese, especially the ripening process, is a lengthy one. With the introduction of process cheese much of this lengthy ripening period has been eliminated. In this generation, the processing of cheese has become a large industry.

1. Process Cheese

Process cheese is the modified cheese made by comminuting and mixing one or more lots of cheese into a homogeneous, plastic mass, with the aid of heat, with or without the addition of water and with the incorporation of not more than 3% of a suitable emulsifying agent. The name process cheese unqualified applies to process Cheddar cheese and those with a varietal name correspond to the process variety indicated by that name. A similar type of cheese is known as *pasteurized cheese* or *pasteurized-blended cheese.*

2. Cream Cheese

Cream cheese has been variously defined as the unripened cheese made by the Neufchâtel process from whole milk enriched with cream, which should contain not less than 65% of fat on the dry basis, and also as the soft, uncured cheese made from curd obtained by the action of either lactic fermentation or rennet or both, on milk enriched with cream. The curd, heated or unheated, salted or unsalted, is drained by gravity and light pressure. The finished product contains not more than 52% of water and, in the water-free state, not less than 65% of milk fat.

Cheese made by the Neufchâtel process is produced from unheated curd obtained by the combined action of lactic fermentation and rennet on whole milk. The curd drained by gravity or pressure is kneaded or worked into a butterlike consistency and is then pressed into forms for immediate consumption or for ripening. The draining is generally done by placing the curd into a cloth or bag. The cloth or bag is changed several times during the draining period which lasts about 4 days.

Process cream cheese may be made by a number of methods one of which is to take plastic cream, condiments, and milk solids, mix them together, homogenize, and pack immediately as cream cheese.

3. Cottage Cheese

Cottage cheese, as defined by the Food and Drug Administration, is the soft uncured cheese prepared as described in the following paragraph. The finished cottage cheese must not contain more than 80% moisture.

Sweet skim milk is pasteurized; calcium chloride may be added in quantity not more than 0.02% (calculated as anhydrous calcium chloride) of the weight of such skim milk; harmless lactic acid producing bacteria, with or without rennet, are added; and the mix is held until it becomes coagulated.

The coagulated mass may be cut; it may be warmed; it may be stirred; and it is then drained. The curd may be washed with water and further drained; it may be pressed, chilled, worked, seasoned with salt.

Creamed cottage cheese is defined as containing not less than 4% by weight of the finished creamed cottage cheese of added milk fat.

X. SKIM MILK

Skim milk is defined as milk from which a sufficient portion of the fat has been removed to reduce the fat content below that of 3.25%. More specifically skim milk is the product obtained in the separation of cream from milk. Davies prefers to differentiate between skim milk and separated milk. The former is obtained by permitting milk to stand and then skimming off the cream and the latter is the product issuing from the skim-milk screw when cream is produced by use of a centrifugal separator.

A representative composition of skim milk is the following: 0.2% fat, 3.5% protein, 5.0% lactose, 0.8% ash, 9.5% total solids, and 90.5% water. The similarity to the composition of buttermilk is evident (see below). Hand-skimmed milk will generally contain more fat than separator-skimmed milk. The fat content of hand-skimmed milk is of the order of 0.75%, otherwise the composition is about the same.

XI. BUTTERMILK

Buttermilk was formerly defined as the product that remains when fat is removed from milk or cream in the process of making butter by churning. If sweet cream is used for churning, the buttermilk produced does not differ from ordinary skimmed milk. The amount of buttermilk produced in the United States is very large because for every 100 lbs. of butter, approximately 166 lbs. of buttermilk are obtained. This means there is available about 4,000,000,000 lbs. of buttermilk yearly. Actually little of this is used for human consumption. Buttermilk contains most of the nutrients of milk except the butterfat.

TABLE 58

COMPOSITION OF BUTTERMILK

Type	Fat, %	Protein, %	Lactose, %	Ash, %	Total Solids, %	Water, %
Genuine[a]	0.5	3.0	5.3	0.7	9.5	90.5
Genuine[b]	0.5	3.5	4.6	0.7	9.3	90.7
Cultured[b]	0.2	3.5	5.0	0.8	9.5	90.5

[a] L. A. Rogers, U. S. Dept. Agr., *Bull.* **319** (1916).
[b] C. Chatfield and G. Adams, U. S. Dept. Agr., *Circ.* **549** (1940).

Most of the buttermilk available commercially for food purposes is cultured buttermilk made by souring pasteurized skimmed or partly skimmed milk, or reconstituted milk made from skim-milk powder and water, by the addition of an ordinary butter starter such as *Streptococcus lactis* or some similar type. Salt is added to skim milk in the proportion of 2 to 4 oz. per 10 gal. of skim milk. The salt improves the flavor and helps to make a smoother, less lumpy buttermilk. The mixture is then pasteurized at 185° F. for 30 min. to produce the highest viscosity, after which it is cooled to 70° F. and inoculated. The incubation or ripening period requires about 12 to 15 hrs. The curdled skim milk is then cooled to 40° F. during which time it must be agitated to assure a smooth, lumpless body. This product may then be turned into "flake" buttermilk by spraying molten butter fat into it. Separation of whey in the finished product during storage is a production problem. Cultured buttermilk is most stable at acidities between 0.87–0.93%. The best storage temperature is 33° F.

It is preferable to limit the maximum fat content in cultured buttermilk to a low value so that people who must be on a fat-free diet may be able to rely on buttermilk as part of that diet.

Condensed Buttermilk

Condensed buttermilk is the semisolid product prepared by evaporating fluid buttermilk, usually in a vacuum pan, until it has a heavy consistency. It contains enough acid to prevent spoilage.

XII. FERMENTED MILK

Milk is fermented in order to preserve it. A number of organisms have been used to ferment milk. One of the most common is *Lactobacillus bulgaricus*. This has been mentioned in connection with the discussion of buttermilk and sour cream (see pages 430 and 421). Buttermilk is a fermented milk.

TABLE 59

COMPOSITION OF FERMENTED MILKS[a]

Product	Fat, %	Protein,[b] %	Lactose, %	Ash, %	Lactic acid, %	Alcohol, %	Water, %
Kefir							
2 days old	3.6	3.1	3.7	0.6	0.7	0.2	...
4 days old	3.6	3.1	2.2	0.6	0.8	0.8	...
6 days old	3.6	3.1	1.7	0.6	0.9	1.1	...
Koumiss							
1 day old	1.2	2.0	1.6	0.35	0.8	2.7	91.4
8 days old	1.1	1.8	0.5	0.35	1.1	2.9	92.1
22 days old	1.3	1.8	0 23	0.35	1.3	3.0	92.1

[a] L. A. Rogers, U. S. Dept. Agr., *Bull.* 319 (1916).

[b] Some denaturation of protein takes place.

Kefir, a product of southeastern Russia and the Caucasus, is a fermented milk made from the milk of various animals principally sheep, goats, and cows. To prepare this drink, a characteristic starter which consists of "seeds" or Kefir grains is used.

Koumiss, another drink which originated in Russia, is a fermented milk made from mares' milk. It is fermented by milk-souring types of bacteria and yeasts. Both Kefir and Koumiss are limpid, slightly acid, and distinctly alcoholic drinks.

Yogurt, a Turkish product, and Matzoon, an Armenian product, are fermented milks which are sufficiently curdled to form a thick, plastic mass, which is decidedly acid and which contains comparatively little alcohol. Similar products are Egyptian Leban, Indian Dadhi and Sardinian Gioddu.

Acidophilus milk is a fermentation product resulting from the careful inoculation of milk with *L. acidophilus* bacteria. It is very similar in appearance to buttermilk.

The composition of some of these products is given in Table 59.

XIII. WHEY

Whey is the serum which remains after the coagulation of the casein when cheese is manufactured. Thus, for instance, about 90 lbs. of whey and about 10 lbs. of cheese are obtained from 100 lbs. of milk in the manufacture of Cheddar cheese. Whey consists principally of the water, lactose, lactalbumin, and most of the ash of the original milk. It has slightly more fat than buttermilk. A representative composition contains 0.3% fat, 1.0% protein, 5.1% lactose, 0.6% ash, 7.0% total solids, and 93% water. The composition may vary considerably, however, depending on the type of cheese manufactured.

A number of products are made from whey. It is the principal source of commercial lactose. If the quantity of whey is large, and the fat is sufficiently high, a butter known as whey butter can be obtained from the resultant cream produced by running the whey through a separator, neutralizing, ripening, and churning. The composition of the butter produced does not differ significantly from ordinary butter.

Two types of cheese can be made from whey. In one type, the lactalbumin is coagulated by use of acid and heat. The curd is then skimmed and pressed. This type is known as Ziger or Ricotta. In the other type, the whey is concentrated by boiling. On cooling, after concentration a brittle mass is formed. This type of cheese is made mainly in Scandinavian countries and is known as Primost or Mysost.

Powdered and condensed wheys are also made from whey. The former contains about 2.5—5% water and 95–98.5% total solids, while the latter contains about 52% total solids. The components are in the relative proportion in which they exist in the original whey.

XIV. DRIED MILK PRODUCTS

Dried milk products are the products resulting from the removal of water from milk and milk products. The principal dried milk products are dried whole milk, dried skim milk, dried buttermilk, dried whey, and dried cream. These products are also known as whole-milk powder, skim-milk powder, buttermilk powder, powdered cream or cream powder, and whey powder. All of them are generally made by use of spray or drum drying methods but whole-milk powder is more commonly prepared by the spray process and buttermilk powder by the drum method. Actually little dried cream is manufactured. The ratios of the components of all of these products are the same as those of the milk or other milk products from which they are prepared. The composition of several of these products is given in Table 60.

TABLE 60

COMPOSITION OF DRIED MILK PRODUCTS

Product	Fat, %	Protein, %	Lactose, %	Ash, %	Total solids, %	Water, %
Whole milk[a]	26.7	25.8	38.0	6.0	96.5	3.5
Skim milk[a]	1.0	35.6	52.0	7.9	96.5	3.5
	1.0	37.0	49.0	9.0	96.0	4.0
Cream[b]	65.1	13.4	17.9	2.9	99.3	0.7
Buttermilk[b]	5.9	38.7	39.9	7.7	98.1	1.9
Malted milk[a]	8.5	14.6	70.7[c]	3.6	97.4	2.6
	7.5	13.0	73.5	3.0	97.0	3.0

[a] C. Chatfield and G. Adams, U. S. Dept. Agr., *Circ.* 549 (1940).

[b] C. H. Eckles, W. B. Combs, and H. Macy, *Milk and Milk Products.* McGraw-Hill, New York, 1936.

[c] Total carbohydrate.

Dry skim milk is identified as the product obtained by drying sweet skim milk. It must not contain more than 5% of water. Dry whole milk must contain not less than 26% milk fat and not more than 5% of water.

XV. MALTED MILK POWDER

Malted milk is the product made by the combination of whole milk with the liquid separated from a mash of ground barley malt and wheat flour, with or without the addition of sodium chloride, sodium bicarbonate, and potassium bicarbonate, in such a manner as to secure the full enzymic action of the malt extract, and by removing water. The manufacture of malted milk powder involves the preparation of a malt-flour infusion by the process of mashing and the evaporation to dryness of the infusion-milk mixture. The ground malt is used to make malt flour. Malted milk should contain 7.5% of butterfat as a minimum and not more than 3.5% water.

The composition of a representative powder is given in Table 60. This powder is used in combination with milk or water to make a beverage, usually by agitation with an electrical mixer, commonly spoken of as malted milk. Often ice cream, cream, and whipped cream are added.

XVI. FILLED MILK

Filled milk is defined by the Filled Milk Act of 1923 as any milk, cream, or skimmed milk whether or not condensed, evaporated, concentrated, powdered, dried, or desiccated, to which has been added, or which has been blended or compounded with, any fat or oil other than milk fat, so that the resulting product is in imitation or semblance of milk, cream, or skimmed milk, whether or not condensed, evaporated, concentrated, powdered, dried, or desiccated. By its terms the Filled Milk Act prohibits the interstate distribution of any combination of milk, cream, or skimmed milk, with any foreign fat or oil so as to resemble or imitate milk or skimmed milk in any form. This legislation was enacted after a Congressional hearing. Congress determined and declared, "that filled milk, as herein defined, is an adulterated article of food injurious to public health and its sale constitutes a fraud upon the public."

A more vicious adulteration of an analogous type is the substitution, not of an edible oil such as lard, or oleomargarine, but the substitution of mineral oil. Instances have been found in which this relatively nutritionally inert material has been incorporated in cheese.

XVII. IMITATION MILK

A type of imitation milk is a mixture made from soybean. One of the methods of making this product is to boil the beans, beat them to a pulp, and then suspend the vegetable protein in water. This process yields a milky mixture which appears to have many of the properties of milk. It is used a great deal by the Chinese. An analogous type of imitation vegetable milk is made from tara or Payo meal.[38]

XVIII. MILK BEVERAGE

A milk beverage or a skim-milk beverage is a food mixture or confection which is prepared from milk or skim milk as the case may be and to which has been added a sirup or flavor consisting of wholesome ingredients. Among the most common of these are chocolate milk and chocolate drink. Chocolate milk is the beverage made by the addition of chocolate or chocolate flavors to whole milk and contains not less milk fat than does whole milk. Chocolate drink or chocolate-flavored drink generally consists of fluid skim milk or skim-milk powder and water to which have been added cocoa powder, sugar, a stabilizer such as a gum or other thickening agent like tapioca flour, and salt.

---

[38] B. F. Feingold, *J. Allergy*, **13**, 488 (1942).

Selected Bibliography

Andrade, J. L., *Estudios sobre la leche*. Caracas, Venezuela, 1940.

Barthel, C., *Untersuchung von Milch und Molkereiprodukten*. Parey, Berlin, 1920

Davies, W. L., *Chemistry of Milk*. Chapman and Hall, London, 1939.

Eckles, C. H., Combs, W. B., and Macy, H., *Milk and Milk Products*. McGraw-Hill, New York, 1936.

Jacobs, M. B., *Chemical Analysis of Foods and Food Products*. Van Nostrand, New York, 1938

Ling, E. R., *Textbook of Dairy Chemistry*. Wiley, New York, 1930.

Mojonnier, T., and Troy, H. C., *Technical Control of Dairy Products*. Mojonnier Bros., Chicago, 1925.

Roadhouse, C. L., and Henderson, J. L., *The Market-Milk Industry*. McGraw-Hill, New York, 1941.

Rogers, L. A., *Fundamentals of Dairy Science*. Chemical Catalog Co., New York, 1928.

Totman, C. C., McKay, G. L., and Larsen, C., *Butter*. Wiley, New York, 1939.

Wilson, G. S., *Pasteurization of Milk*. Arnold, London, 1942.

"Food and Life," U. S. Dept. Agriculture, *Yearbook of Agriculture*, Washington, D. C., 1939.

Consumer's Guide *Milk Glossary for Consumers:* **6**, No. 19, 6 (1940); **7**, No. 1, 8 (1940), **7**, No. 2, 10 (1940); **7**, No. 3, 14 (1940); **7**, No. 5, 12 (1940); **7**, No. 6, 11 (1940); **7**, No. 8, 13 (1941); **7**, No 9, 14 (1941); **7**, No. 10, 14 (1941); **7**, No. 11, 9 (1941); **7**, No. 12, 11 (1941); **7**, No. 13, 14 (1941), **7**, No. 14, 15 (1941).

Chapter XIII

MEAT AND MEAT PRODUCTS

WALTER M. URBAIN
Chicago, Illinois

It is impossible, in the space allotted in this treatise, to discuss thoroughly the vastness and complexity of the meat-packing industry and its products. What can be discussed, therefore, are only the principal features of interest to the food technologist, and the reader is referred to the given references and to the "Selected Bibliography" at the end of this chapter for works covering particular phases in greater detail.

I. THE RAISING AND MARKETING OF LIVESTOCK

Meat animals considered here are cattle, hogs, and sheep. While meat animals are raised in all parts of the United States, they are more numerous in some sections than in others. The chief regions of animal production are the Corn Belt area of the Mississippi Valley and the range lands of Texas and of the West.

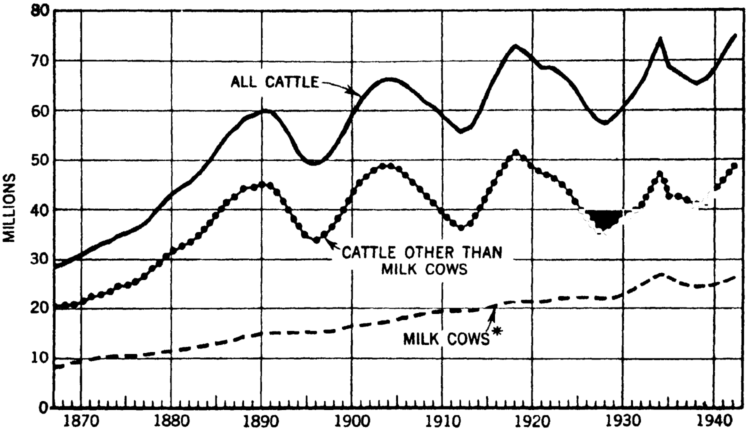
The populations of the three chief classes of meat animals, cattle, hogs, and sheep, in the United States over a period of years are shown in the accompanying charts taken from publications of the Bureau of Agricultural Economics of the U. S. Department of Agriculture (Figs. 28, 29, and 30).

Meat animals of a given species may exist in a variety of breeds. Breeding has played an important role in meat production, since by proper breeding animals exhibiting certain desirable features have been favored to the exclusion of less desirable ones. Thus Fig. 31 (*a*) shows the typical razor-back hog, a range type, a forest-fed hog. Contrasted with it in Fig. 31 (*b*) is a typical hog of the Corn Belt, an obviously better animal from a meat standpoint.

The following are the principal breeds of the chief meat animals, along with the important characteristics desired.

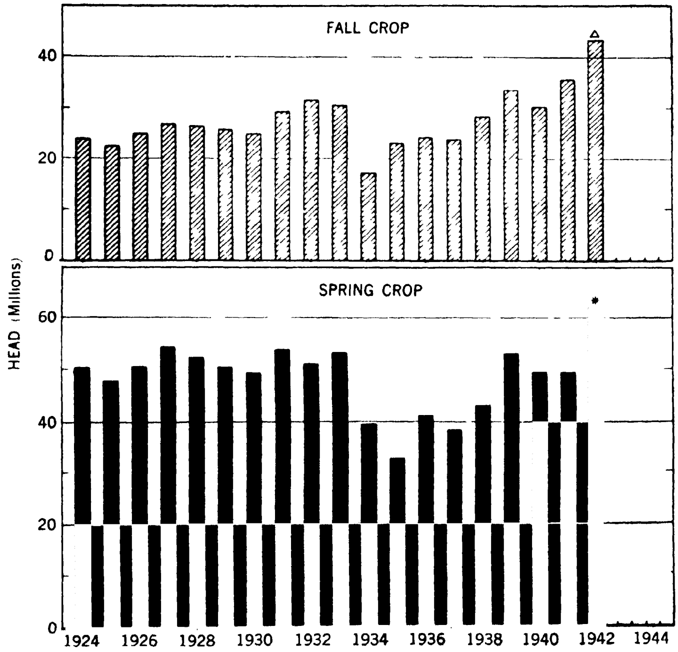
1. Cattle Breeds

One very important quality of cattle raised for beef purposes is their ability to mature at an early age. Preference is given cattle which are short legged, low set, compact with good muscular development in the back, ribs, loins, and thighs, and which have a high ratio of flesh to bone. The chief breeds are Herefords, Shorthorns, Galloways, and Aberdeen Angus.



*Cows and heifers 2 years old and over kept for milk
Data for 1942 are preliminary

Fig 28.—All cattle: number on farms January 1, United States, 1867 1942 [1]



* Preliminary
Δ Based on number of sows indicated to farrow in fall of 1942 and average number of pigs saved per litter during the past 5 years

Fig. 29 —Spring and fall pig crops. United States, 1924 1942 [2]

---

[1] "The Livestock Situation," U. S. Dept. Agr., Bur. Agr. Economics, February (1942), p 1.

[2] "The Livestock and Wool Situation," U. S. Dept. Agr., Bur. Agr. Economics, July (1942), p 1

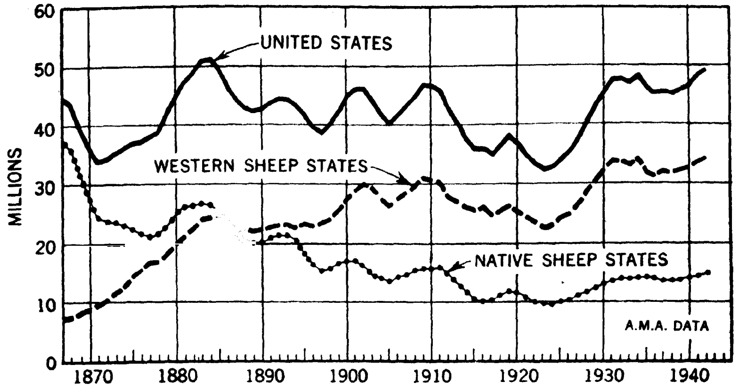


Fig. 30.—Stock sheep and lambs: number of farms January 1, 1867–1942.[3]

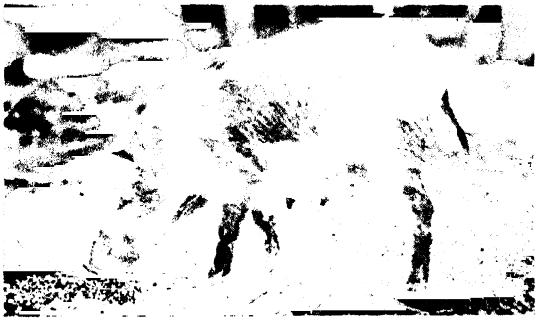


Fig. 31 *a*.—Razor-back hog.[4]

Fig. 31 *b*.—Middlewestern hog.

---

[3] "The Livestock Situation," U. S. Dept. Agr., Bur. Agr. Economics, March (1942), p. 1.

[4] Photograph of Razor-back hog obtained from the Bur. Animal Ind., Agr. Research Adm., U. S. Department of Agriculture.

2. Pig Breeds

In the United States, hogs are primarily of the butcher type; that is, they produce other cuts in addition to bacon. They are compact, low set, and well fleshed. The most widely used breeds are Poland Chinas, Berkshires, Duroc-Jerseys, Chester Whites, and Hampshires.

3. Sheep Breeds

Sheep and lambs serve as sources of both meat and wool, and the two purposes are not completely capable of the fullest realization in one type of animal. The most widely known breeds are Cheviots, Lincolns, Southdowns, Merinos, Rambouillets, Oxfords, and Shropshires.

Livestock may be marketed through a public stockyard. More than 60 central livestock markets have been established in this country, the principal ones of which are in the Middle West. Shipments are made by train or truck. These markets usually include stockyards, pens, sheds, and enclosures to accommodate the stock Animals are purchased by competitive bidding. They may also be sold directly to meat packers without going through a central market.

II. THE OPERATIONS OF THE MEAT-PACKING INDUSTRY

Basically, meat packing embodies six classes of operations: (*1*) buying the livestock; (*2*) dispatching of the animals; (*3*) dressing of the carcasses; (*4*) preparation of the meat and meat products from the carcasses; (*5*) preparation of the meat by-products; (*6*) selling the meat and by-products. Frequently one meat-packing plant may carry out only some of these operations, leaving others to other plants where facilities may be provided for what are specialized operations. This is particularly true in the preparation of the by-products of the meat-packing industry.

It is apparent that the efficient operation of the industry depends upon ease of intercommunication among a number of units. The location of meat-packing plants, therefore, is chosen with care, both with regard to source of materials and market for the products. The tendency has been to locate meat-packing plants near the source of supply and to do less of the slaughtering operations in the consuming areas.

As indicated above, meat-packing plants do not follow a definite pattern. Their facilities vary greatly according to the amount and kind of processing they do. There are, however, usually associated with them certain features which may be described as follows:

A meat-packing plant consists usually of holding pens or shelter sheds, etc.; a killing floor, on which the stock is usually both dispatched and dressed; a refrigeration chamber for removing body warmth from the meat; various chilled workrooms for cutting the carcass into trade cuts; specialized rooms for such operations as curing, sausage making, smoking, etc. In addition, the meat-packing plant is likely to have associated with it facilities for the manufacture of lard, tallow, grease, and other fat products; gelatin, glue, animal feeds, and other secondary by-products as well as facilities for the handling of such items as hides, skins, and pelts. Also there are such important units as an adequate steam-power plant, refrigeration plants, chemical control laboratories, maintenance shops, and other departments of a service nature. Further, there are the administration departments, including the buying and selling organizations. A few of the national companies usually have well-staffed research laboratories, which are essential units for progress.

III. STATISTICS ON MEAT PRODUCTION AND CONSUMPTION

The meat-packing industry is one of the most important industries in the United States. The per capita consumption of meat is available through the figures of the Bureau of Agricultural Economics of the U. S. Department of Agriculture. Table 61 shows this for periods since 1925.

TABLE 61

PER CAPITA CONSUMPTION OF MEATS IN THE UNITED STATES,[a] IN POUNDS

Item	1925–1929	1936–1940	1939	1940	1941
Beef and veal	62.3	63.5	61.8	62.5	70.8
Lamb and mutton	5.4	6.7	6.7	6.6	7.0
Pork	68.2	61.3	64.4	72.5	70.2
Total	135.9	131.5	132.9	141.6	148.0

[a] "The Livestock Situation," U. S. Dept. Agr., Bur. of Agr. Economics, January (1942), p. 13.

Perhaps a better idea of the fundamental importance of the industry can be gained more easily by examination of Table 62, which shows the production of meats in the United States.

TABLE 62

PRODUCTION OF MEATS IN THE UNITED STATES[a]
Average per year, in million pounds

Item	1925–1929	1936–1940	1939	1940	1941
Beef and veal	7,270	8,112	7,962	8,177	9,198
Lamb and mutton	643	887	876	878	928
Pork	8,480	8,161	8,627	9,920	9,570
Total	16,393	17,160	17,465	18,975	19,696

[a] "The Livestock Situation," U. S. Dept. Agr., Bur. of Agr. Economics, January (1942), p. 13.

It is the experience of the industry that the production of meat varies with the national income. Usually, however, there is a lag in this relation because of the time required to increase the number of meat animals. The per capita consumption of meat varies with the income of the individual. Table 63 shows this relation.

TABLE 63

PER CAPITA CONSUMPTION, IN POUNDS, OF FAMILIES AND SINGLE INDIVIDUALS IN VARIOUS INCOME GROUPS[a]

Meat	Under $500	$500–$1000	$1000–$1500	$1500–$2000	$2000–$3000	$3000–$5000	$5000 and over
Beef	21.4	36.1	45.4	53.4	57.7	62.2	77.5
Veal	3.0	3.7	5.6	7.0	8.5	9.9	12.6
Lamb and mutton	0.8	2.1	3.7	5.8	8.6	12.4	25.7
Bacon and salt side	31.8	24.3	21.5	21.1	21.1	23.1	25.5
Other pork	17.8	24.7	32.9	34.9	39.0	41.9	45.9
Other	8.5	13.8	16.3	16.7	16.4	12.9	11.9
Total	83.3	104.7	125.4	138.9	151.3	162.4	199.1

[a] "The National Food Situation," U. S. Dept. Agr., Bur. of Agr. Economics, July (1942), p. 20

Several factors operating at the time of the outbreak of the second World War have increased the production of meat. The industry, therefore, has been able better to meet the extra demands occasioned by the armed forces, by export to the United Nations, and by increased domestic demand attributed to a marked rise in the national income. Rationing of meat for civilian use has been necessary in order to limit the domestic demand. Table 64, by way of illustration, shows the increase of Federal Government purchases of one class of meats, canned pork, occasioned by the war effort.

TABLE 64

FEDERALLY INSPECTED PRODUCTION AND GOVERNMENT PURCHASES OF CANNED PORK FOR SPECIFIED PERIODS,[a] 1936–1942

Period, monthly av.	Federally inspected production, million lbs.	Government purchases, million lbs.
1937	9.7	. .
1938	11.5	. .
1939	16.9	. .
1940	23.5	. .
1941		
January	30.6	. .
February	27.7	. .
March	29.9	. .
April	30.7	15.0
May	36.6	28.1
June	37.6	6.6
July	42.1	20.5
August	41.8	28.9
September	36.4	23.9
October	38.9	48.5
November	44.4	37.7
December	66.1	20.8
1942		
January	76.3	75.3
February	74.0	41.7
March	77.6	57.5
April	81.4	107.8
May		85.0

[a] "The Livestock and Wool Situation," U. S. Dept. Agr., Bur. of Agr. Economics, June (1942) p. 7

IV. BUREAU OF ANIMAL INDUSTRY DEFINITIONS

Since the meat-packing industry is one primarily concerned with the processing of a food material, it is subject to the regulation of certain governmental agencies. In addition to State and municipal agencies, the Federal Government through the Bureau of Animal Industry[4a] of the Department of Agriculture exerts a control on meat processing. Among the functions of the B. A. I., as it is known, is the establishment of definitions of meat and meat products. A few of the more important definitions given by the Bureau are as follows:[5]

"1. **Carcass:** All parts, including viscera, of a slaughtered animal that are capable of being used for human food.

"2. **Primal parts:** The usual sections, cuts, or parts of the dressed carcass commonly known in the trade, such as sides, quarters, shoulders, hams, butts, bellies, beef tongues, beef livers, before they have been cut, shredded or otherwise subdivided preliminary to use in the manufacture of meat products.

"3. **Meat products:** Any edible part of the carcass of any cattle, sheep, swine, or goat, which is not manufactured, cured, smoked, processed or otherwise treated.

"4. **Fresh meat:**[6] The word 'fresh' shall not be used on labels in connection with any meat or products the ingredients of which, in whole or in part, have undergone any process of curing."

These definitions give the exact meaning of the terms. To a large extent they are the result of common practice. For instance, the term "meat products" is defined by the B. A. I. to include the edible parts of the carcass and this is without a doubt an accepted usage. The limiting phrase "which is not manufactured, cured, smoked, processed, or otherwise treated" restricts the B. A. I. meaning to what is ordinarily regarded as fresh meat, *i. e.*, meat which is subjected only to the dressing operations. Cured meats, typified by ham or bacon, receive an additional processing known as curing, which is not directly defined by the B. A. I. Curing is a process subject to great variations of procedure, formula, and end results. The definitions of the B. A. I. are necessary for enforcement of their inspection and have done much to provide useful standards for the industry.

V. CLASSES AND GRADES OF MEAT

Meat is largely the voluntary muscle of the carcasses of cattle, sheep, and swine. In addition to these variations of species, differences within a species in sex, age, condition, and heredity also govern the character of the meat. Depending upon certain characteristics, meat of a given species may be of good, mediocre, or inferior quality. There is, therefore, need of a system of grading. In the following discussion the important systems of grading meat in use in the United States are described.

[4a] In 1943, the Meat Inspection Division of the Bureau of Animal Industry was transferred to the Food Distribution Administration of the U. S. Department of Agriculture.

[5] Regulations Governing the Meat Inspection of the U. S. Dept. Agr., B. A. I. Order 211, Revised, November 1, 1922, pp. 2 and 3.

[6] Regulations Governing the Meat Inspection of the U. S. Dept. Agr., B. A. I. Order 211, Revised, November 1, 1922, p. 36.

Two definitions are needed. By *class* is meant the sex condition of the animal. Thus by class designation, information is given, for instance, as to whether a male animal was castrated or not. Since it is necessary for the animal to reach sexual maturity before sex differences become noticeable, the question of class is not important for calves, for instance, but is so for beef. By *grade* is meant the designation by which quality, frequently within a class, is described.

Each species has its own grading system in order to take into account the characteristics of the individual species and the methods of marketing. The importance of grading makes it advisable to understand the systems for grading beef, lamb, and pork in detail.

1. Beef

There are two separate systems of grading beef, the meat most susceptible to variation in quality as far as the consumer is concerned. These two systems are the "Institute" or "Packer" grades and the "U. S." grades. Both systems grade beef on the basis of conformation, finish, and quality. The designations of each system are different: numbers in the Institute system, names in the U. S. system. The Institute system describes the grade of the animal in greater detail and is more commonly used in trading. The consumer seldom sees beef designated in terms of the Institute grades, except as they are reflected in the "brand" grades by which the individual meat packer sells his meat. The Institute grades exist primarily to assist the meat packer in his operations. The U. S. grades, on the other hand, are aimed at telling the consumer the quality of the animal as a food. The Institute system includes reference to the class of the animal; the U. S. system does not. There are five classes of beef animals as follows:

1. Steer, a male castrated while very young.
2. Heifer, a young female that never has had a calf.
3. Cow, a mature female which has borne a calf.
4. Bull, a mature male.
5. Stag, a male castrated after reaching maturity.

There are ten Institute grades and seven U. S. grades of beef. References should be made to the accompanying chart for the comparison of the grades of the two systems. Details of each grade are as follows.

U. S. Prime Grade—Institute 0

Only beef from steers or heifers can fall into this grade and the animals are usually less than 3 years old. Certain features of the carcass are important, the back fat should be one-half to three-quarters of an inch thick; "marbling" (lean interspersed with fat) must be present.

At present, during the war, this grade has been combined with U. S. choice grade.

U. S. Choice Grade—Institute 1 and 2

Only a small difference from the higher grade may place beef in this category. Too much or too little fat may grade it down.

U. S. Good Grade—Institute 3

Greater age variation is permitted. Too much or too little fat may make an otherwise "Prime" grade only a "Good."

U. S. Commercial—Institute 4 and 5

This grade has fair quality and is the grade most widely encountered. Fat covering is small and is usually colored yellow; marbling is seldom present.

U. S. Utility—Institute 6 and 7

The animals of this class usually have bony carcasses and little fat covering, which is yellow.

U. S. Cutter—Institute 8

Cows make up most of the animals of this grade. The flesh is very dark.

U. S. Canner—Institute 9

This is the lowest grade and is usually made up of old cows

BEEF GRADING SYSTEM. U. S. D. A AND AMERICAN MEAT INSTITUTE GRADE STANDARDS FOR STEER, HEIFER, AND COW BEEF[a]

Institute Grades Steers and Heifers	U. S. D. A. Grades All Classes	Institute Grades Cows
0	Prime	
1	Choice	
2		
3	Good	1
4	Commercial	2
5		3
6	Utility	4
7		5
8	Cutter	6
		7
9	Canner	8
		9

[a] R. B. Hinman and R. B. Harris, *The Story of Meat.* Swift & Company, Chicago, 1942, p. 91.

2. Veal

Because the animals are young, class differences in veal are unimportant. There are five U. S. grades of veal: choice, good, commercial, utility, and cull.

3. Sheep

There are two main groups based on age: lamb and mutton. Sheep reach maturity at 12 to 14 months and it is at this age that the change from lamb to mutton occurs. Lambs constitute 90% of the sheep marketed for meat.

There are two general market classes of lambs: (*1*) the "genuine spring lamb," a lamb from 3 to 6 months of age, and (*2*) "spring lamb," which are lambs up to one year of age and are usually marketed in the fall and winter.

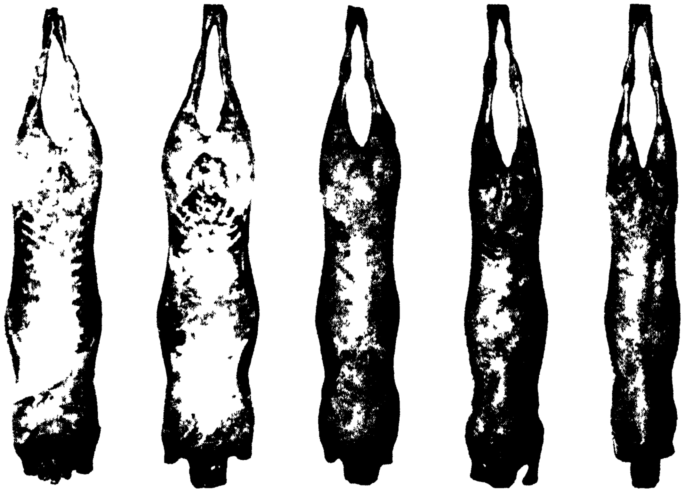


Fig. 32.—The five grades of lamb [7]
From left to right: Prime, Choice, Good, Commercial, Utility.
(Courtesy of the Food Distribution Administration.)

There are six grades of lamb: Prime, choice, good, commercial, utility, and cull. Five of these grades are illustrated in Figure 32. It should be mentioned, however, that at present, during the war, the prime grade has been combined with choice, making five grades of lamb.

4. Pork

Most of the pork reaching the market comes from hogs 7 to 12 months old. Hence, differences of sex are not important. Nevertheless, there are classes of hogs, as follows: barrows, unsexed young males; gilts, young females; sows, mature females; boars, mature uncastrated males; and stags, castrated mature males.

There are three grades of pork, designated simply; one, two, and three.

---

[7] Robert B. Hinman and Robert B. Harris, *The Story of Meat.* Swift & Company, Chicago, 1942, p. 101.

The use of the systems for grading meat has been affected by the second World War. The U. S. grades for beef have replaced the Institute grades for the present, and beef grading is done almost entirely by Department of Agriculture graders. Similar procedures have been followed in the grading of lamb.

VI. COMPOSITION OF MEAT

Since meat is the result of a complex organic development, it may be expected that its composition is complex. Like most living tissue, its chief component is

Table 65

Composition of Beef[a]

Sample	Inedible portion, %	Edible portion			
		Water, %	Protein, %	Fat, %	Ash, %
Carcass, thin	19	66	18.8	14	0.97
Carcass, medium	16	60	17.5	22	0.87
Carcass, good	15	55	16.3	28	0.79
Wholesale cuts:					
Chuck, thin	19	71	19.2	9	0.94
Chuck, medium	17	65	18.6	16	0.88
Chuck, fat	15	60	17.6	22	0.82
Chuck, very fat	13	52	15.0	32	0.74
Loin, thin	16	64	18.6	16	0.95
Loin, medium	14	57	16.9	25	0.84
Loin, fat	12	53	15.6	31	0.77
Loin, very fat	10	44	12.8	43	0.62
Round, thin	12	71	19.7	8	1.00
Round, medium	11	67	19.3	13	0.95
Round, fat	10	63	18.7	17	0.90
Round, very fat	9	58	17.4	24	0.82
Rump, thin	27	60	17.4	22	0.88
Rump, medium	24	53	15.5	31	0.77
Rump, fat	22	48	14.2	37	0.69
Rump, very fat	19	40	11.4	48	0.56

Table 66

Composition of Pork[a]

Sample	Inedible portion, %	Edible portion			
		Water, %	Protein, %	Fat, %	Ash, %
Carcass, thin	18	50	14.1	35	0.8
Carcass, medium	12	42	11.9	45	0.6
Carcass, fat	10	35	9.8	55	0.5
Wholesale cuts:					
Belly, thin	9	43	11.4	45	0.6
Belly, medium	7	34	9.1	56	0.5
Belly, fat	6	25	6.6	68	0.4
Loin, thin	24	63	17.9	18	1.0
Loin, medium	19	58	16.4	25	0.9
Loin, fat	16	52	14.8	32	0.8
Ham, thin	18	60	17.2	22	0.9
Ham, medium	14	53	15.2	31	0.8
Ham, fat	11	46	13.2	40	0.7

[a] Charlotte Chatfield and Georgian Adams, "Proximate Composition of Food Materials," U. S. Dept. Agr., *Circ.* **549** (1940).

water. Next in importance is a class of compounds containing nitrogen and grouped under the term protein. There are numerous other components including fats, minerals, vitamins, acids such as lactic, glycogen, etc. Meat, naturally, is not of fixed composition. Variations occur with species, breed, sex, age, and condition.

TABLE 67

COMPOSITION OF LAMB[a]

Sample	Inedible portion, %	Edible portion			
		Water, %	Protein, %	Fat, %	Ash, %
Carcass, thin	31	66.3	17.1	14.8	0.9
Carcass, medium	22	55.8	15.7	27.7	0.8
Carcass, fat	19	46.2	13.0	39.8	0.7
Wholesale cuts:					
Leg, thin	23	71.0	18.4	9.1	1.0
Leg, medium	17	63.7	18.0	17.5	0.9
Leg, fat	16	59.8	16.7	22.4	0.8
Loin, thin	23	. .	. .	.	.
Loin, medium	15	. .	. .	.	.
Loin, fat	12	.		.	
Rib, thin	35	65.3	17.7	15.6	0.9
Rib, medium	21	51.9	14.9	32.4	0.8
Rib, fat	18	38.7	11.2	49.2	0.6
Shoulder, thin	27	67.2	16.7	14.7	0.9
Shoulder, medium	20	58.3	15.6	25.3	0.8
Shoulder, fat	18	51.3	13.6	34.3	0.7

TABLE 68

COMPOSITION OF VEAL[a]

Sample	Inedible portion, %	Edible portion			
		Water, %	Protein, %	Fat, %	Ash, %
Carcass, thin	23	71	19.7	8	1.0
Carcass, medium	21	68	19.1	12	1.0
Carcass, fat	19	65	18.5	16	0.9
Wholesale cuts:					
Chuck, thin	22	73	19.9	6	1.1
Chuck, medium	20	70	19.4	10	1.0
Chuck, fat	18	67	19.0	13	1.0
Loin, thin	19	71	19.7	8	1.0
Loin, medium	17	69	19.2	11	1.0
Loin, fat	16	65	18.6	15	1.0
Hindquarter, thin	21	71	19.7	8	1.0
Hindquarter, medium	19	68	19.1	12	1.0
Hindquarter, fat	17	65	18.5	16	0.9

[a] Charlotte Chatfield and Georgian Adams, "Proximate Composition of Food Materials," U. S. Dept. Agr., *Circ.* **549** (1940).

There are, however, certain ranges characteristic of given types, and in Tables 65–68 are given the results of analyses indicating these ranges for the four major classes of components, water, protein, fat, and mineral (ash). The analysis will yield results depending upon the type of sample and in listing the composition it has, therefore, been necessary to include a description of the part of the carcass sampled. For the interest of the consumer the composition is shown for the edible portion. The inedible part of the portion purchased is shown in per cent.

VII. POST-MORTEM CHANGES IN MEAT

1. Rigor Mortis

A period of time usually elapses between the dispatching of an animal and the consumption of a part of the carcass as food. Normally the length of much of this period is governed by the necessities of commercial practice in making meat available to the consumer. In addition, however, there may be deliberately occasioned periods of aging, which have as their purpose the improvement of quality particularly as to tenderness and flavor.

After the carcass is dressed, it is chilled. During the chilling period there is a solidification of the fat, and approximately simultaneously there is the development of *rigor mortis*. *Rigor mortis* is a condition in which the muscles harden and shorten. The phenomenon is accompanied with the formation of lactic acid and other acids (from the tissue glycogen) with the generation of heat. After reaching a maximum in approximately 24 hrs. there is a slow diminution of the rigor, and the muscles again become soft and flaccid. At refrigeration temperatures, the changes which follow *rigor mortis* are slow.

2. Aging of Meat

Lean meat is essentially voluntary muscle tissue. These muscles, through their connecting tendons, act upon the bone structure of the animal by contraction and thus accomplish the desired movements. Muscles are made of a mass of fibers bound together by a connective tissue composed of the protein collagen. The amount of connective tissue in a given muscle is largely governed by the activity of that muscle. Muscles of less activity have less connective tissue. Age and sex also are factors. It is generally considered that the more connective tissue present, the tougher is the meat.

Each muscle fiber consists of an elongated sac containing the muscle protein material. The sac (sarcolemma) is comprised of an elastic material forming a sheath. During *rigor mortis* the contents of this sac lose their transparency, and the sheath its elasticity. The whole muscle fiber contracts longitudinally. Following the maximum development of *rigor mortis*, there is a gradual breakdown of the protein material within the muscle sac resulting in the muscle as a whole again becoming soft. Prolonged storage will cause a gradual disintegration of the proteins, including the connective tissue. Meat aged to this degree has been *tendered*.

The change of the microscopic structure of veal with time is shown in Fig. 33. The structural changes are best interpreted as a breakdown (autolysis) of the original organization of the tissue.

The rate of tendering is a function of the temperature. Usually meat is held just

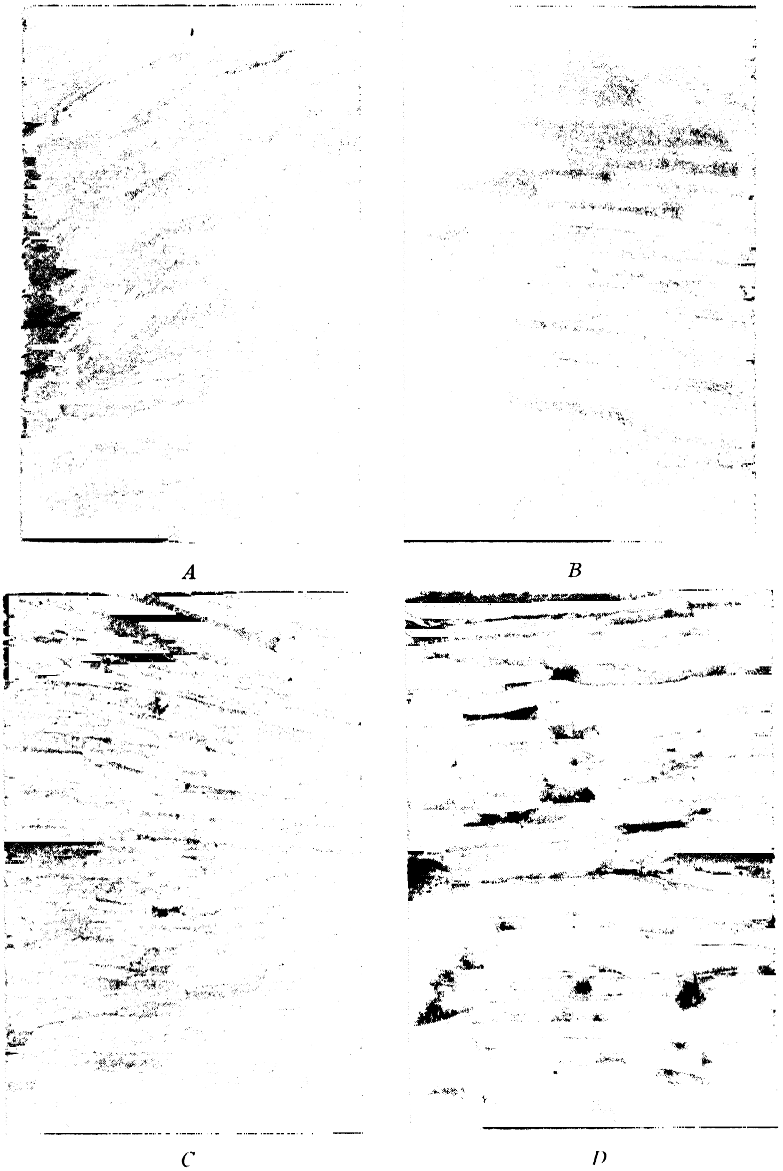


Fig. 33 -The effect of aging on the microscopic structure of veal.[8] *A* Six hours after slaughter. *B*. Three days after slaughter. *C*. Six days after slaughter. *D*. Thirteen days after slaughter.

---

[8] The photomicrographs are the work of Dr. C. H. Koonz of the Research Laboratories of Swift & Company, Chicago, Ill.

above the freezing point in order to discourage microbial growth. In normal practice, most meat is consumed without being purposely aged. Only certain choice cuts of beef are intentionally aged. Aging is an expensive process, not only because of the expenses involved in holding the meat an extra long period under refrigeration, but because of certain other changes which occur in the meat:

Shrinkage.—The cut muscle surfaces lose water continuously by evaporation. To a certain extent, this loss may be overcome by raising the humidity of the holding room.

Loss of Bloom.—*Bloom* is a word describing the appearance of the surface and is related to the condition of the pigments, connective tissue, and fat. Loss of desirable bloom occurs during aging.

Microbial Growth.—Despite low temperatures, molds and bacteria make some progress on the surfaces, necessitating trimming of these areas.

3. Dark Cutting Beef

A small percentage of the cattle received at the meat-packing plant cut "dark" when the meat is dressed. It is impossible to determine in advance which cattle will cut dark. Such beef, as far as it is known, is equally as nutritious as others and, when cooked, cannot be distinguished from normal beef. Because of its color in the fresh state, however, it is discriminated against in retail trade, and must therefore be sold for less than its normal value.

Dark cutting beef occurs in young cattle as frequently as or oftener than in old. It is not to be confused with the darkness that is occasioned by age and sex, which is due mainly to a higher concentration of pigment. Dark cutting beef is due to a condition of the animal which alters the state of the pigments. The pigment myohemoglobin occurs naturally in two forms in muscle tissue: (*1*) as oxyhemoglobin on the surface, and (*2*) as reduced hemoglobin in the interior. The former is a bright red and lends to beef its characteristic bright color; the latter is a dark purple and is the cause of the dark appearance of a freshly cut surface of beef. As the reduced hemoglobin acquires oxygen on exposure to air, the surface assumes its more normal bright red color. In dark cutting beef, the pigment remains in the reduced state and fails to acquire its normal complement of oxygen, even on the surface. As a consequence the beef is dark in color.[9] A second characteristic of dark beef is a stickiness easily ascertained by touching it.

Much work has been done to determine the cause of the condition which results in dark beef. Many theories, such as the method of dispatching the stock, delayed bleeding, inheritance, feeds, excitement, etc., have been advanced. A real lead, however, was obtained by a group working under the National Livestock and Meat Board, who found that the *p*H of dark beef is appreciably higher than normal and that such tissue was depleted of muscle sugar. To some extent dark cutting beef could be produced experimentally by injections of insulin into healthy animals. This and other evidence led to the association of dark cutting beef with a disturbance of the sugar metabolism of the animal. Just what the nature of this disturbance is, is not understood, but it does provide some explanation for the phenomenon.

---

[9] D. L. Mackintosh and J. L. Hall, "Some Factors Related to the Color of Meat," Am. Soc. Animal Production Rec. Proc. 28th Ann. Mtg. 1935, pp. 281–286.

The depletion of the tissues of sugar decreases the amount of lactic and other acids capable of being produced after death. The increase in *p*H resulting from this lack of acid disturbs the normal equilibrium between oxygen and hemoglobin in meat and shifts it in the direction of having a predominance of the reduced form. The "sticky" condition of the beef can also be associated with the relatively high *p*H.

Dark cutting beef, therefore, is the result of a particular post-mortem change in beef which can be related to some abnormal condition in the living animal.

VIII. PRESERVATION OF MEAT

Meat is highly perishable, and in the time which intervenes between dispatching and the use of meat as food, protection must be provided against several factors of spoilage. The chief factors which operate to produce spoilage are: (*1*) microorganisms such as bacteria, molds, and yeasts; (*2*) air, producing oxidative changes or dehydration; (*3*) light acting catalytically in the oxidative changes attributed to air; (*4*) enzymes, which can cause autolysis of the protein tissue or hydrolysis of the fats and other changes. In some instances, limited action by any one of these factors, which ultimately can cause spoilage, produces changes usually regarded as desirable, *e. g.*, the mild autolysis of the protein which occurs in aging and tendering. Of the factors listed as involved in meat spoilage, the action of microorganisms is without question the most important. Bacteria can cause many types of spoilage, some of which will be taken up in detail later. The presence of large numbers of bacteria causes off-flavors, discolorations, slime, etc., and ultimately decomposition. Molds develop into objectionable visible growths and produce undesirable flavors and odors. The destructive action of air is usually limited to the production of discolorations, rancidity development in the fatty tissue, frequently with the assistance of a catalyst such as light or bacteria, and to dehydration. Light fades certain pigments and promotes rancidity. Enzymes, naturally present in the tissue, cause the hydrolytic changes mentioned above.

Control of the factors listed here constitutes preservation of meat. Through the ages there have been developed various methods of controlling some or all of these factors in order that meat may be preserved for some period to suit the convenience and need of man. Perhaps the most universal method of preservation has been that of refrigeration. Lowering of the temperature slows down microbial growth and, if the temperature is low enough, may virtually prevent it. The effects of the other spoilage factors are also minimized by low temperatures. Salting of meats is an effective method of controlling microbial growth. Drying is very effective. Smoking accomplishes drying and at the same time adds to the meat certain compounds contained in the smoke which exert a preservative action. The use of certain chemicals such as borax is effective in preservation, but, generally speaking, the employment of such materials is not practiced in this country. Canning of meats, as with other foods, is a common method of preservation.

In some of the sections which follow, certain of these methods of preservation will be taken up in greater detail, since from both volume and technological points of view they are very important.

IX. FREEZING OF MEAT

In the United States only about 1% of the commercially marketed meat (not including poultry, see page 533) has been frozen and thawed prior to sale. Freezing

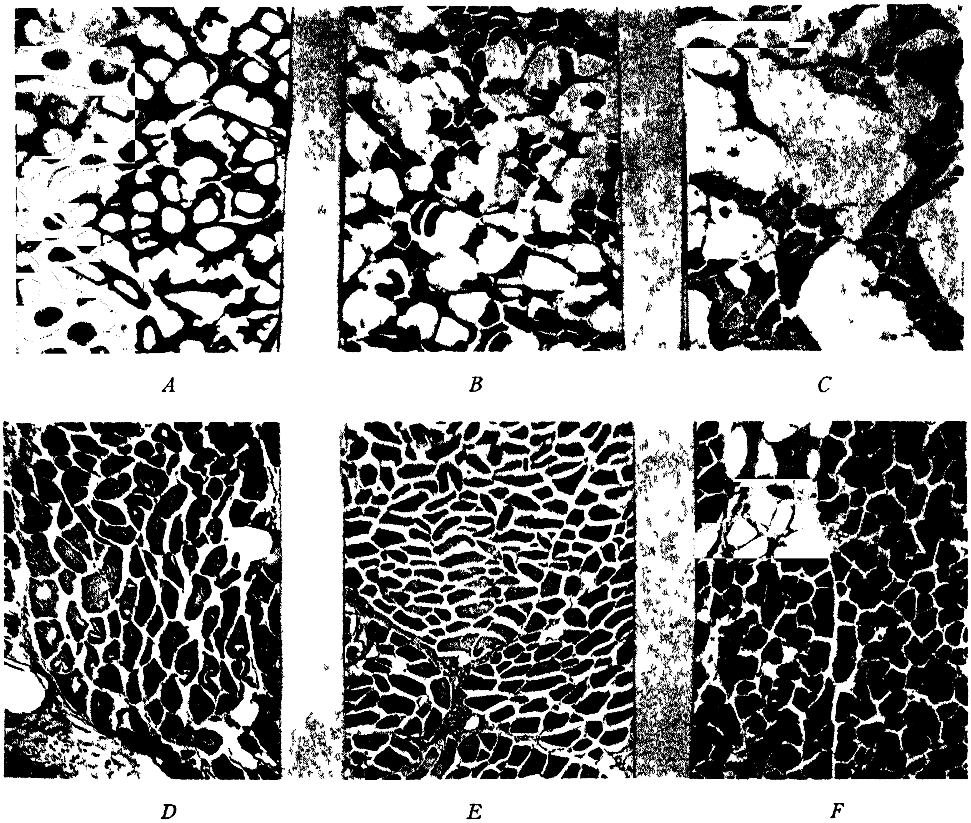


Fig 34 —The effect of freezing temperatures (freezing rates) upon the microscopic structure of beef[10] *A*. Frozen at −45 6° C *B* Frozen at −23 3° C *C* Frozen at −6 7° C *D* Frozen at −45 6° C and defrosted at 10 0° C *E* Frozen at −6 7° C and defrosted at 10 0° C *F* Unfrozen

and thawing produce some microscopic changes which affect the physical properties of the meat.

---

[10] J M Ramsbottom and C. H Koonz, *Food Research*, **4**, 425 (1939)

The major changes are associated with the movement of moisture. Ice formation may occur in different ways, depending on the method of freezing. The size, number, and distribution of ice formations are governed by the rate of freezing. If meat is frozen slowly, water will leave the fibers and tends to be frozen outside of them; if frozen rapidly, the water will be frozen within the fibers. The rate of freezing in the air will be governed largely by the temperature of the air and the size of the cuts. Upon defrosting the meat the effects of the rate of freezing become apparent. Drip, a watery fluid which exudes from the lean, is larger in the case of the slowly frozen meat. The microscopic picture of defrosted muscle is very similar to that of the unfrozen muscle.

Other factors affect the amount of drip. The more cut surface on the muscle, the more liquid water may exude before being absorbed and the consequent greater drip.[10] The greater the time between dispatching and freezing, the greater the amount of drip.[11] Likewise, the longer the freezer storage period, the greater the drip. The temperature of storage does not appear to affect the amount of drip.[12]

X. CURED MEATS

Meat curing was a process originally aimed at the preservation of meat without refrigeration. Its primary feature was the addition of salt to the meat, but through the centuries there have been added variations and combinations with other processes so that today a cured meat product may not only be salted, but also smoked and/or dried, sugar-cured (added sugar), treated with sodium nitrite and/or sodium nitrate in order to "fix" the color, *i. e.*, render the color heat-stable, spiced and aged to improve flavor. Cured meat may be ground or comminuted, or it may consist of certain cuts, such as hams, bellies (bacon), brisket (of beef), leg muscles (dried beef). In general, meat curing is confined to beef and pork.

The need for preservation of meat with salt is less in this day of refrigeration. Meat is cured today principally because the various cured meat products have made for themselves an important place in our daily diet. Certain dishes have become "American Customs," *e. g.*, bacon or ham and eggs for breakfast. In the days of little or no refrigeration it was necessary to use relatively high concentrations of salt in order to obtain preservation. Today, however, salt is used primarily for the flavor it adds and secondarily for its preservative action. As a consequence, there has been a tendency to reduce the concentration and the favoring of "mild cures." In this reduction, the careful control over sanitation and refrigeration possible in a modern meat-packing house has played a major role; for it is not sufficient that proper conditions for preservation of the product be maintained after the product leaves the meat plant or market, but they must also be carefully maintained during the processing. The analysis of typical modern hams is shown in Table 69.

---

[11] J. M. Ramsbottom and C. H. Koonz, *Food Research*, **5**, 423 (1940).

[12] J. M. Ramsbottom and C. H. Koonz, *Food Research*, **6**, 571 (1941).

As a consequence of this tendency to reduce the salt, there is a need for the realization that the cured meats of today require refrigeration for preservation. Education of all those who handle cured meats is necessary, and the proper refrigeration of such products as hams and bacon throughout the time they are held until they are used for food, assures the consumer of a product which is of good quality.

TABLE 69

ANALYSIS OF CURED AND SMOKED PORK HAMS[a]

Type product	Moisture, %	Salt, %	Sodium nitrite, p. p. m.	Sodium nitrate, %	Sugar, %
"Tender" smoked	69.5	3.45	49	0.025	0.82
"Tender" smoked	68.8	3.77	17	0.045	0.44
"Tender" smoked	68.8	4.12	22	0.096	0.68
"Tender" smoked	68.0	3.05	57	0.083	0.62
"Tender" smoked	66.5	4.18	29	0.027	0.80
"Tender" smoked	68.7	4.98	93	0.014	0.57
Av.	68.4	3.89	45	0.048	0.65

[a] C. E. Gross, "What Is a Ham," *Proceedings* of the Chemistry and Operating Section, American Meat Institute, Chicago, 1939, p. 13.

The regulations of the B. A. I. permit the utilization of five curing agents (other than spices) in the curing of meat: salt, sugar, sodium nitrate, sodium nitrite, and vinegar.[13] The introduction of these materials into the meat plus the concomitant processing constitute the curing process. Basically, there are two types of curing processes, the first involving the addition of the curing agents in the dry state and the second in the form of a brine. These basic procedures are subject to great variations, depending upon the type of product being cured. A comminuted product, such as sausage, requires merely the mixing of the dry curing agents in order to obtain a good distribution. A pork ham may be cured by applying the dry curing-agent mixture to the surface, and through the relatively slow process of diffusion, fairly good distribution may be obtained. Or the ham may be immersed in a water solution of the curing agents (pickle) and the process of diffusion accelerated and distribution improved. The modern way of curing ham involves the pumping of the solution of curing agents through the vascular system of the ham (vein pumping) and in this way obtaining rapid and good distribution of the curing agents.

Of the five officially permissible curing agents, four are of major importance and are the ones employed in most meat curing. They are salt, sugar, sodium nitrate, and sodium nitrite. Salt is primarily a preservative and flavoring agent. Sugar is largely added for its flavor, although not exclusively, as will be shown. Sodium nitrate and sodium nitrite are color-fixation agents. Sodium nitrate is also a bacteriostat.

---

[13] U. S. Dept. Agr. Amendment 4 to B. A. I. Order 211, Revised, October 19, 1925.

The pigments of meat are principally the conjugated proteins hemoglobin and myohemoglobin. The former is present in amounts depending upon the quantity of residual blood in the tissue. The latter is an integral part of the tissue. The two pigments are closely related chemically and differ in their reactions largely by degree rather than by kind. The absorption spectra are practically identical, and, consequently, their colors are also identical. For the purpose of discussion of these pigments we shall refer to hemoglobin only; essentially, the same statements will be true of myohemoglobin.

In fresh meat, hemoglobin may exist in two forms, as the compound hemoglobin, itself, or as oxyhemoglobin, a compound of hemoglobin and molecular oxygen. The first compound exists only in the absence of oxygen; the second requires the presence of oxygen. The conversion of one into the other is reversible and involves an "oxygenation" rather than an oxidation. In both compounds the iron of the heme portion of the molecules is in the ferrous condition. Normally, only the pigment of the surface of the meat exists in the form of oxyhemoglobin. In the interior, the oxygen demands of the biological processes of the tissue use up the oxygen, and since no fresh supply is accessible because there is no circulation of blood, only the unoxygenated form exists.

When either hemoglobin or oxyhemoglobin is heated, coagulation of the protein occurs. Simultaneously, the color changes. Oxyhemoglobin is red; hemoglobin is purple. The heated compound, hematin, is brown. On cooking, fresh meat turns brown. In some instances where the concentration of hemoglobin is low, the fact that the heated compound has poor pigment properties causes a lightening of the color to a gray or cream, as in pork or veal. Hematin observed in sufficient concentration is brown.

When cured meats are cooked, however, they retain their redness. This is due to the fixation of the color by sodium nitrate and sodium nitrite. The chemistry of the reactions whereby this heat stability of color is obtained has been studied. Sodium and potassium nitrate were the first color fixation agents used. It was discovered, however, that the nitrates were merely sources of nitrites, and in 1927 the B. A. I. permitted the use of nitrites directly. The use of nitrates was continued by the industry, since it was found that proper color fixation could be obtained only in certain limited cases by the use of nitrite alone and that the presence of nitrate in addition was usually desirable. In general, best results are obtained by a combination of the two curing agents.

Sodium nitrite is a source of nitric oxide, which is the real color fixative. The color reactions of the curing process may be given as follows:

$$
\begin{aligned}
&\mathrm{NaNO_3} + \text{reducing conditions} \longrightarrow \mathrm{NaNO_2}\\
&\mathrm{NaNO_2} + \text{acid conditions} \longrightarrow \mathrm{HONO}\ \text{(nitrous acid)}\\
&\mathrm{HONO} + \text{reducing conditions} \longrightarrow \mathrm{NO}\\
&\mathrm{NO} + \mathrm{Hb}\ \text{(hemoglobin)} \longrightarrow \mathrm{NO\cdot Hb}\ \text{(nitric oxide hemoglobin)}
\end{aligned}
$$

The pigment of uncooked cured meat is nitric oxide hemoglobin. This compound is an analogue of oxyhemoglobin and involves merely the substitution of a molecule of nitric oxide for one of oxygen. The absorption spectra of the two compounds are very similar and, as a consequence, so are the colors (Fig. 35). Upon heating, nitric oxide hemoglobin is converted to nitric oxide hemochromogen, which also is pink, in contrast to the brown of hematin derived from hemoglobin or oxyhemoglobin.

In the reactions given for the color-fixation process, it should be noted that two conditions are necessary for the proper development of nitric oxide hemoglobin: reducing and acid conditions. The conversion of sodium nitrate to the nitrite is a process of reduction, normally accomplished by bacterial action. This bacterial reduction is a relatively slow process and is dependent upon the fortunate circumstances of the presence of the right kind of bacteria to accomplish the reduction and upon the right conditions for their growth. Prior to the use of sodium nitrite, much off-colored cured meat could be ascribed to "undercure" resulting from failure to obtain bacterial reduction of the nitrate used in the cure.

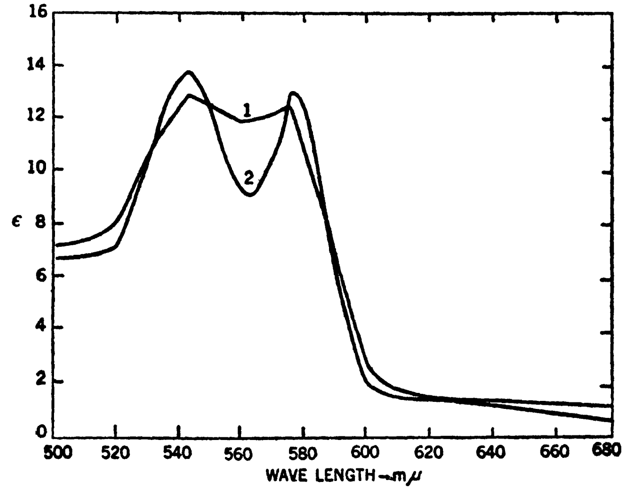


Fig. 35.—The absorption spectra of nitric oxide hemoglobin (curve 1) and of oxyhemoglobin (curve 2).[14]

The second necessary condition, namely, one of acidity, is one admirably met by the natural conditions of the meat. Sodium nitrite, which exists only in alkaline or neutral solutions, is not a ready source of nitric oxide. On the slightly acid side, between *p*H 5.5 to 6.4, it decomposes to yield nitric oxide comparatively easily. This is the normal *p*H range for meat, and consequently is the *p*H range in which the curing reactions proceed. Below a *p*H of 5.5, there is too rapid decomposition of the nitrite (or nitrous acid), and if curing conditions should develop such a degree of acidity, the odor of nitric oxide becomes noticeable. In addition, there is a reaction of the pigment to yield principally a true oxidation product, methemoglobin or ferrihemoglobin. In this compound the iron is in the ferric state. It is brown, and consequently undesirable in cured meats.

---

[14] L. B. Jensen and W. M. Urbain *Food Research,* **1,** 263 (1936).

Nitric oxide, in the presence of oxygen, reacts with any of the ferrous hemoglobin compounds. If they are compounds such as oxyhemoglobin or nitric oxide hemoglobin, the end product is methemoglobin. Consequently, there is further reason for the maintenance of reducing conditions during the cure. Even where sodium nitrite is the starting curing material and no conversion of nitrate to nitrite is required, reducing conditions remain an absolutely necessary condition to the development of good cured color.

Of the curing agents regularly employed, one, namely sugar, contributes to the setting up of reducing conditions.[15] Ordinarily, cane or beet sugar is employed, although in recent years there has been an increasing amount of corn sugar used for meat curing. The use of the latter is limited in some products by the fact that it caramelizes at a lower temperature and tends to darken the meat on cooking. Sugars, *per se*, even the so-called reducing sugar (*e. g.*, corn sugar), do not contribute to the reducing conditions. Bacteria, however, act upon them and produce unknown substances which exert a marked reducing action. Since corn sugar is primarily a monosaccharide and consequently more easily available to bacteria than the disaccharide sucrose, it usually exerts its action more rapidly than sucrose. Any advantage of corn sugar would, therefore, lie only in short cures and would be lost if the curing period were sufficiently long to permit utilization of sucrose by bacteria. In fact, the use of corn sugar in large amounts in long cures is virtually prohibited by the vigorous microbial growth it promotes, which usually causes an acid fermentation and consequent lowering of the *p*H to a point where color destruction occurs.

The stability toward oxidation of the cured meat pigments is different from that of the fresh meat pigments. That such differences should exist is easy to understand from the chemical compositions of the two classes of pigments. In general, atmospheric molecular oxygen is the principal oxidizing material to which the pigments are exposed. In the case of the fresh meats, there are two pigments, but only one is capable of oxidation, namely, the unoxygenated hemoglobin. Exposure of this compound to oxygen can cause two reactions to occur:

$$\mathrm{Hb} + \mathrm{O_2} \rightleftarrows \mathrm{HbO_2} \qquad (1)$$

$$\mathrm{Hb} + \mathrm{O_2} \longrightarrow \mathrm{Met\ Hb} \qquad (2)$$

The first reaction is merely the oxygenation reaction. It is a reversible reaction, dependent upon the pressure of the oxygen. The second reaction is the true oxidation reaction and usually is not reversible, although if the proper conditions are applied reduction of methemoglobin to hemoglobin can be accomplished. For the maximum rate for the second reaction, there exists an optimum oxygen pressure. This has been found to be about 20 mm. of oxygen pressure and is the pressure[16] which permits a sufficient concentration of oxygen to accomplish the oxidation, but insufficient to convert more than half of the pigment to oxyhemoglobin. It should be noted that this optimum pressure is very much less than the normal oxygen pressure of air (approximately 152 mm.). The oxidation of the pigments of fresh meat, therefore, increases with lower oxygen pressure and complicates the storage of meat in inert gases, such as nitrogen, unless virtually complete removal of oxygen is accomplished.

---

[15] D. A. Greenwood, W. Lee Lewis, W. M. Urbain, and L. B. Jensen, *Food Research*, **5**, 625 (1940)

[16] James M Neill and A. Baird Hastings, *J. Biol. Chem.*, **63**, 479 (1925).

Exposure of nitric oxide hemoglobin to oxygen gives rise to the following reaction:

$$O_2 + NO{\cdot}Hb \longrightarrow Met\ Hb + NO_2$$

This reaction is not reversible, and there exists no optimum oxygen pressure for it. It appears that the rate of this reaction is proportional to the oxygen pressure.[17] It has a high-temperature coefficient and is accelerated by a lowering of the *p*H. Light accelerates the air oxidation.

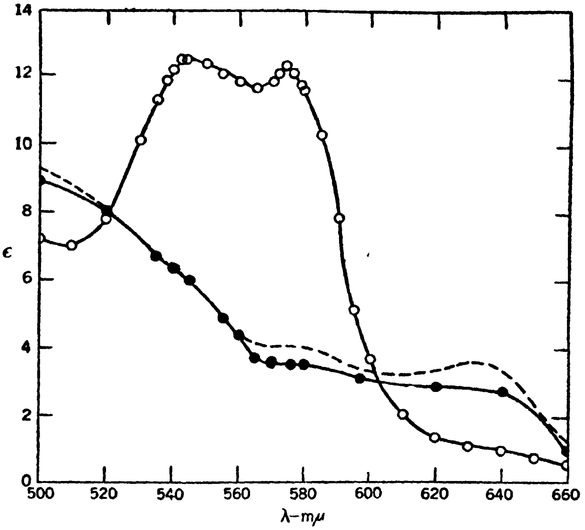


Fig. 36.—Conversion of nitric oxide hemoglobin to methemoglobin by oxygen.[17] O–O NO·Hb, *p*H 7.2, before oxidation. ●–● After oxidation. - - - Methemoglobin, *p*H 7.2.

Figure 36 shows the change in absorption spectrum of nitric oxide hemoglobin occasioned by the conversion to the brown pigment methemoglobin by action of atmospheric oxygen. The data of Table 70 show the effects of *p*H and temperature upon the rate of oxidation by atmospheric oxygen of solutions of nitric oxide hemoglobin.

---

[17] W.M. Urbain and L. B. Jensen, *Food Research*, 5, 593 (1940).

It is apparent, therefore, that as a meat pigment, nitric oxide hemoglobin is not perfect. Its heated analogue, nitric oxide hemochromogen, is probably less stable, but owing to its insolubility in water it is less reactive and apparently more stable. Brown discolorations are important and present serious problems in such products as sliced bacon, where relatively large surface areas are exposed to atmospheric oxygen.

There exist other discolorations which are characteristic of cured meats. Certain oxidizing substances and hydrogen sulfide can form green compounds from nitric oxide hemoglobin. The occurrence of these oxidizing compounds and hydrogen sulfide in meat is due to bacterial action. Normally, it is possible to distinguish between the oxidizing compounds and hydrogen sulfide in meat. A discoloration caused by action of an oxidizing agent of bacterial origin usually exists well below the surface of the meat. One due to hydrogen sulfide, on the other hand, exists only in the surface, and frequently by cutting a fresh surface, one can see what may appear to be a normal color change rather quickly to a green. The explanation for this latter phenomenon probably lies in the fact that the hydrogen sulfide has combined with the pigment to form hydrogen sulfide hemoglobin (purplish compound) which upon contact with oxygen is converted to a green compound.[14]

TABLE 70

AIR OXIDATION OF NITRIC OXIDE HEMOGLOBIN[a]

*p*H	Temperature, °C.	% oxidation after					
		1 day	2 days	3 days	6 days	13 days	29 days
5.75	0	58	57	58	80	95	88
5.75	10	53	68	76	88	103	102
5.75	37	102	100	100	105	104	96
6.75	10	28	61	69	91	106	100
8.25	0	3	3	3	4 5	7	44
8.25	10	5	18	22	52	73	78
8.25	37	70	...	...	...	...	...

[a] W. M. Urbain and L B. Jensen, *Food Research*, 5, 593 (1940)

The identity of these green compounds is not known. Figures 37 and 38 show the absorption spectra for green hemoglobin derivatives obtained by the action of bacterial oxidizing agents upon nitric oxide hemoglobin and of hydrogen sulfide upon oxyhemoglobin. It is probable that the pigments of the green discolorations of cured meats are related to those that gave rise to these spectra. The spectrophotometric results indicate the existence of a continuous reaction in both cases and it is probable that the discolorations observed in meat are not to be associated with a single pigment.

One rather bizarre manifestation of the green discolorations is the formation of green rings in frankfurts, bologna, dry sausage, meat loaves, and other cured comminuted products. Here, too, oxidizing and sulfide-forming bacteria are responsible for the formation of a green pigment, which occurs in the form of a ring or zone inside the product. The formation of this ring is apparently due to the existence of an optimum oxygen tension which occurs at some depth from the surface. The oxygen

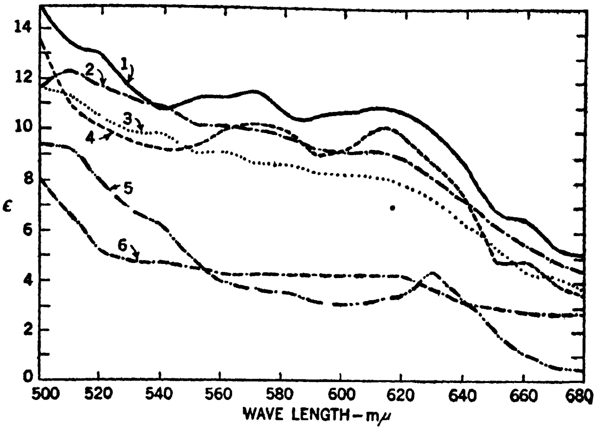


Fig. 37.—Spectra obtained by action of bacterial oxidizing enzymes on nitric oxide hemoglobin; also spectrum of methemoglobin.[14] Curves 1, 2, 3, and 4, green derivatives. Curve 5, methemoglobin, *p*H 5.9. Curve 6, yellow derivative obtained by long exposure of nitric oxide hemoglobin to bacterial enzymes.

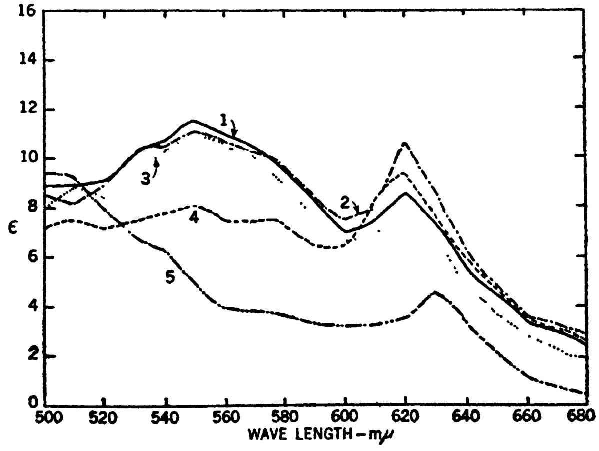


Fig. 38.—Spectra obtained by action of hydrogen sulfide on oxyhemoglobin; also spectrum of methemoglobin.[14] Curves 1, 2, and 3, green derivatives obtained by fifteen minutes' exposure to hydrogen sulfide. Curve 4, spectrum obtained by permitting solution giving curve 2 to stand 20 hrs. Curve 5, methemoglobin, *p*H 5.9.

tension decreases from a maximum at the surface of the product (*e. g.*, a frankfurt) to essentially zero at the center. In the intervening distance there is a gradient of oxygen tension, and at the zone of green formation there exists the tension which is an optimum for the conversion of the cured meat pigment to the green compounds. If the proper bacterial products are present, greening results. Jensen[18] has summarized the conditions for the development of green rings as follows:

"The oxidizing reactions of bacteria are due to (*1*) R-O_2 thermolabile oxidizing enzyme insensitive to catalase; R-O_2 thermostable oxidizing enzyme insensitive to catalase and both types sensitive to catalase; (*2*) H_2S or sulfide-forming bacteria, both aerobic or facultative anaerobes growing in an optimum zone in the sausage; (*3*) oxidizing bacteria growing in an optimum zone only; and (*4*) preformation of sulfhemoglobin, oxidizing to a green pigment at optimum and maximum O_2 tensions."

Since bacteria are always associated with green discolorations, it is obvious that proper sanitary measures are the most direct methods of prevention.

Another problem of major importance in cured meat manufacture that is bacterial in nature is "ham sours" or bone taint. This problem has become less acute in recent years "due to a natural progress in the industry" and to "a specific advance in curing methods."

According to Jensen,[19] there have been observed seven types of ham souring:

1. "Shank sour" or sour tibial marrows.
2. "Body sour" or sour meat, not marrows. (Also called "loin sour.")
3. "Aitchbone sour" or souring of the *os pubis* remaining in the ham.
4. "Stifle-joint sour" or souring of area around articulation of femur and tibia-fibula.
5. "Body-bone sour" or sour femur marrow.
6. "Butt sour" or sours in butt between aitchbone and muscle.
7. "Puffers" or a gaseous condition, no longer encountered.

The cause of the sour condition is bacterial action upon the tissues. Much effort has been expended to determine just how the bacteria make their way to the rather inaccessible places in which the souring occurs. In general, Jensen and Hess[20] concluded that the bacterial invasion of tissues of ham occurs during the dying period and after death. Any bacteria which can grow at 0 to 3.3° C. (curing cellar temperatures) and in marrows and which are salt-tolerant can cause any kind of souring. Food-poisoning bacteria are not found in sour hams.

Sours in the quick cured hams, *i. e.*, vein pumped, have been largely prevented by bleeding hogs properly, adequate refrigeration, sawing for sealed-shank marrows, prompt handling, bacteriologically controlled pickle, and strict general sanitation.

XI. SAUSAGE

Sausage usually consists of a comminuted meat, to which may be added spices, water, dried skim milk, or other ingredients, all of which are contained in a casing.

---

[18] L. B. Jensen, *Microbiology of Meats.* Garrard, Champaign, 1942, p. 162.

[19] L. B. Jensen, *loc. cit.*, p. 131.

[20] L. B. Jensen and W. R. Hess, *Food Research*, **6**, 273 (1941).

The chief meats employed in making sausage are beef and pork. Sausage may be made of fresh or cured meats. It may be smoked or not, and it may or may not be cooked in the packing plant. Casings are usually of animal origin, but in recent years some sausage has been enclosed in synthetic coverings made of regenerated cellulose.

There are many varieties of sausage, frequently of local character. In the trade there are three general classifications of sausage: (*1*) fresh sausage, uncooked, made of fresh meat and unsmoked; (*2*) smoked and cooked sausage; and (*3*) dry or summer sausage.

XII. CANNED MEATS

Canned meat products can be grouped into two classes: (*1*) those which are composed of cuts canned whole, such as hams, and (*2*) those which are composed of comminuted meats. By far the larger portion of meat normally canned falls into the latter class. Meats are canned primarily for preservation without refrigeration and consequently the majority are heated sufficiently high to sterilize them. A few, however, are canned with a processing schedule which is not sufficient to prevent microbial action and such products must, therefore, be kept under adequate refrigeration. Whole hams are an example of the latter class. The principal reason for not cooking such products sufficiently to permit storage without refrigeration is that the amount of cooking required is such that the product would not retain its form and normal palatability.

Canned meat products include ham, corned beef, corned-beef hash, luncheon meats, chopped ham, pork shoulder, Vienna-type sausages, and potted meats. The second World War has added sliced bacon (cooked) and slab bacon (uncooked). Such products are for export to the United Nations and for use by the armed forces.

An export product, not canned, but properly mentioned here since it is designed to be held without refrigeration, is the asphalt-coated ham or bacon. These products are intended for export to tropical countries. They usually consist of products cured with extra amounts of salt and smoked heavily. They are wrapped in several thicknesses of paper and then dipped in asphalt. When this substance hardens they are well protected against moisture, dirt, vermin, etc., and suitable for export into countries where refrigeration is not available. The asphalt coating may be removed easily.

XIII. DEHYDRATED MEATS

The invasion of Malay and the East Indies by Japan in 1941 and 1942 cut off the chief supply of tin for the United States. Under the Federal Surplus Commodities Corporation (Lend-Lease), this country was called upon to supply enormous quantities of food to the United Nations, particularly to Great Britain. The shortage of shipping caused by increased demand was further exaggerated by the German U-boat program. The net result of all this has been a decided impetus in the development of dehydrated foods, including meat.

Meats which have been successfully dehydrated on a commercial scale are beef and pork, although there is nothing in the process of dehydration which should limit it to only these meats. The meat must first be cooked and ground. The dehydrating process is usually an air-drying process and both tunnel and rotary driers are employed. Vacuum drying, also, has been used. The product is dried to 10% moisture. Several per cent of salt may be added if desired. Excessive fat content causes some difficulty, and usually the meat is trimmed quite lean. Because of the drying, however, the percentage of the fat increases and may be as high as 40% in dehydrated pork. Rancidity development in the fat may be inhibited by vacuum sealing of the cans or by the use of an antioxidant such as gum guaiac. In packaging the product, it is usually compressed in cans so that the maximum weight of product is contained in a given space. Figure 39 illustrates the saving in shipping space as the result of dehydration and compression of fresh beef.

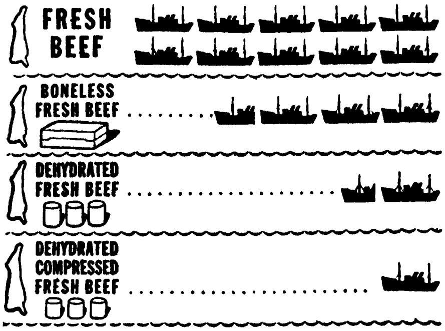


Fig. 39.—Saving in shipping space occasioned by the dehydration and compression of fresh beef.[21]

XIV. MEAT COOKERY

Meat is cooked largely to kill certain microorganisms naturally associated with it and to render it more palatable.[22] There are several ways to accomplish the necessary heating, and wrapped in this fundamental process are all the arts of culinary skill. As a general rule, meat should be cooked in such a way as to retain its nutritive value.

One noticeable effect of cooking meat is the coagulation of the proteins. This denaturation of the protein is accompanied by a firming of the meat, increase in density, and by shrinkage. The collagen of the connective tissue is converted to gelatin and tenderness may be increased. The meat pigments are coagulated and if the meat is fresh (not cured), the color changes from red to brown or gray. The degree to which all these effects occur depends upon the amount of heat and the way it is applied.

---

[21] Courtesy *Meat Magazine*, Chicago, Ill.

[22] For an excellent discussion of meat cookery see Belle Lowe, *Experimental Cookery*. 2nd ed, Wiley, New York, 1937.

Broadly speaking there are two ways to cook meat: (*1*) by dry heat and (*2*) by moist heat. In general, dry heat is used for the more tender cuts and moist heat for those less tender. The cooking temperature is important. Low-temperature cooking generally results in a smaller cooking loss, greater juiciness, more uniformity of cook, and requires a longer time. In general meat cooked at low temperature does not brown as attractively as meat cooked at higher temperatures.

The conditions for cooking the varieties of cuts and kinds of meat vary considerably. In addition, the individual preferences of the consumers govern the conditions of cooking. In general one may summarize the factors involved as follows: (*1*) the cooking temperature; (*2*) the total weight of the cut and its surface area; the shortest distance to the center or its thickness; (*3*) the degree of cooking desired, whether well done, medium, or rare; (*4*) the composition of the meat (proportion of fat and lean); (*5*) the "ripeness" of the meat, since the more aged the meat the shorter the cooking time, a phenomenon which is probably related to the effect of the structure upon the rate of heat transfer.

Older cooking directions specified cooking times in terms of a cooking temperature and "minutes per pound." Much reliance was placed on the experience of the cook. Where possible, today cooking directions are given in terms of internal temperatures measured by means of a meat cooking thermometer inserted in the center of the cut, combined with cooking temperatures. To a large extent the consumer judges the degree of cooking a cut has received by the color. This is not an exact measure, but, in general, rare meat is reddish, medium shows some pink, and well done is gray or brown.

Beef may be served rare, medium, or well done. Veal and pork are always served well done. Lamb is usually well done, but chops are not infrequently served medium. Much of the choice as to the degree of cooking lies with the individual. Fresh pork, however, must always be well done (heated to a minimum temperature in all parts of 58.3° C.) in order to kill the organism *Trichinella spiralis* which is responsible for trichinosis. It is estimated that about 1% of hogs are infested with this parasite, and since there is no known practical method of detecting its presence in pork, it is a consumer responsibility to cook pork sufficiently to guarantee its destruction. Pork is usually heated well above the thermal death point of *Trichinella spiralis,* normally to about 85° C.

Rough internal meat temperature ranges for the accepted methods of meat cooking are as follows:

Beef	
Rare	60° C.
Medium	71° C.
Well done	77° C.
Pork	
Well done	85° C.
Lamb	
Medium	80° C.
Well done	83° C.
Veal	
Well done	82° C.

XV. NUTRITIONAL ASPECTS OF MEAT

Meat is a source of four important classes of dietary components: protein, fat, minerals, and vitamins. Meat is a protective or foundation food, essential to the dietary as a source of high-quality proteins, rich in phosphorus and iron and an excellent source of the vitamins of the B complex.

The chief proteins of meat are albumin and myosin.

In the growing or developing organism, protein is essential for the building of protein tissue. The organism cannot by itself synthesize certain amino acids and must therefore ingest them. In this way there has arisen a list of certain amino acids essential for growth, and the protein capable of supplying all of them in sufficient quantities is described as "complete." The proteins of meat are "complete."

In both the growing and mature organism there is another function of protein which is essential to life, namely, the replacement of nitrogenous compounds used in metabolism and tissue repair. Certain proteins can supply amino acids of sufficient variety and amounts to permit maintenance, although they would not be satisfactory for growth. These proteins are termed "partially complete."

There is a final physiological function of protein, namely, to supply energy. Any protein not needed for growth or maintenance may be "degraded" by removal of the nitrogen in the form of urea, which is excreted in the urine, and then utilization of the residue for energy in a fashion similar to that by which sugars and fats are metabolized.

The optimum amount of protein in a diet seems to vary with the purpose to which it is put. According to Sherman,[23] 10 to 12% in the form of protein of the total calories consumed are all that are required either for growth or maintenance under ordinary conditions. Anything above this may be used for energy directly. As a concentrated source of protein, meat ranks high and supplies all direct protein needs as well as supplementing the less complete vegetable and cereal proteins.

The other dietary components obtainable from meat—fats, minerals, and vitamins—although secondary to the protein content, are none the less important. Fats are a prime source of energy and supply the essential fat acids—linoleic and arachidonic. The minerals phosphorus, iron, copper, sulfur, sodium, chlorine, and iodine are present in sufficient amounts to make meat an important source of them. Meat is a most important dietary source of thiamine (B_1), riboflavin, niacin (nicotinic acid), and other members of the B complex vitamins. Vitamins A and C are also present in meats.

XVI. FOOD POISONING RESULTING FROM MEAT

Food poisoning (see page 381) resulting from eating meat is almost exclusively of bacterial origin. According to Jensen,[24] there are four kinds of bacteria which produce food poisoning in the human subject: *Staphylococcus* (*S. aureus* and *S. albus*), *Salmonella, Clostridium botulinum*, and *Streptococcus* (*S. viridans*).

---

[23] Henry C. Sherman, *Chemistry of Food and Nutrition.* 5th ed., Macmillan, New York, 1937, p. 230.

[24] L. B. Jensen, *Microbiology of Meats.* Garrard, Champaign, 1942, p. 233 *et seq.*

Of these the commonest is *Staphylococcus*. This organism is very widespread but only a few strains can produce gastrointestinal symptoms in man. It poisons by producing an enterotoxin, of an unknown, although possibly a carbohydrate nature, which is usually destroyed by adequate cooking. Symptoms (vomiting and diarrhea) usually appear in 2 to 3½ hrs. after ingestion.

The second organism responsible for food poisoning in meat, *Salmonella*, does not form a toxin, but acts through infection of the bowel directly. Symptoms appear in from 10 to 72 hrs. or longer, depending upon the number of organisms ingested. This organism is easily killed at pasteurizing temperatures. Fatalities are rare.

Clostridium botulinum does not grow in air. Nitrates tend to inhibit its growth. It forms a neurotoxin which paralyzes the central nervous system. Symptoms appear in from 1 to 4 days after ingestion. Mortality is high. The organism is easily killed by cooking, being destroyed in 30 sec. to 5 min. at 80° C. Botulism is a very rare disease, and foods infected with the organism have an "indescribable cheesy stench" so that they would not be regarded as edible.

The streptococci are rarely responsible for food poisoning in meats. They form no toxin but attack the bowel directly. These bacteria are killed at pasteurizing temperatures.

XVII. FOOD AND FEED BY-PRODUCTS OF MEAT

In this section are included the main by-products of meat packing, those which may be used by man as food, others prepared for animal feeding, and still others which are used only for the latter purpose.

1. Fancy Meats

It was not very long ago that liver was considered of little value in the diet. Since the discovery of the role of liver in the control of anemia, there has been developed an appreciation of the value of a class of meat products similar to liver, in that they are not voluntary muscle, but organs. Recent studies have shown that kidneys, hearts, sweetbreads, and brains are nutritionally superior to meat in some respects. Their production is becoming an important item of the list of the products of meat-packing plants.

2. Meat Fats

Fats, oils, and greases are the most important of the by-products of the meat-packing industry. There are many varieties of these, depending upon the source and method of preparation. Some are defined as edible; others are not. Only those which are edible are of concern here. Limitations of space prevent an adequate description of these products and only the barest summary of their properties can be given.

All fats are compounds formed by combination of glycerol and fat acids. The type or combination of types of fat acids determines the characteristics of the fat. Fats which tend to be soft or liquid (oils) contain fat acids either of short-length carbon chains or of relatively long chains which are unsaturated, such as linoleic or oleic acid. Harder fats contain the more saturated fat acids such as palmitic or stearic.

For a meat fat to be regarded as edible it should meet certain requirements as to source, preparation, and storage. In general, edible fat is obtained from all fat which is not contaminated with offal during dispatching and dressing of the animal carcass. Certain fundamentals of preparation, such as promptness of rendering, method, and degree of refining, are also important. Finally, if the fat is to be stored, conditions and the period of storage must be such that edibility is not destroyed by deterioration.

In the trade, several general properties are used to determine the quality of fat and the use to which it may be put:

Color (governed by type of fat, method of preparation, and refining).
Odor and flavor (governed by type of fat, method of preparation, refining, and state of oxidation or rancidity development).
Moisture (chiefly related to the method of preparation).
Free fat acid (a measure of the hydrolysis to which the fat has been subjected).
Hardness, as indicated by the melting point or titer of the fat acids (related to the type of fat acid present, whether long or short, saturated or unsaturated).
Iodine number (degree of unsaturation of fat acids).
Initial peroxide value (state of oxidation).
Saponification number (molecular weight of fat acids).
Unsaponifiable matter (nonfat constituents).
Keeping quality (ability to resist rancidity development).

Some of the properties of fats can be altered by processing. Refining purifies. It can be accomplished by adsorption of impurities with materials such as fuller's earth, bleaching carbons, etc.; by alkali refining which removes mainly fat acid by formation of soap and which can be removed by settling, centrifuging, or washing with water. Deodorization, which is primarily a low-pressure steam distillation, removes volatile constituents such as fat acid, odoriferous materials, etc. Chilling and settling separate high- and low-melting constituents. Hydrogenation saturates double bonds and raises the melting point.

The chief meat fats and their distinguishing characteristics are:

Lard.—Lard is defined by the Bureau of Animal Industry as follows:[25] "The fat rendered from fresh clean, sound, fatty tissues from hogs in good health at the time of slaughter, with or without lard stearin or hardened fat. The tissues do not include bones, detached skin, head fat, ears, tails, organs, wind-pipes, large blood vessels, scrap fat, skimmings, settlings, pressings and the like and are reasonably free from muscle tissue and blood."

---

[25] U. S. Dept. Agr. Amendment 13 to B. A. I. Order 211, Revised August 13, 1940.

Lard is a relatively soft fat having a characteristic flavor. Its color is quite light. Moisture is usually under 1%, and free fat acid should be below 0.5%. The fat acids of lard are principally stearic, palmitic, lauric, myristic, oleic, and linoleic. Lard is not very resistant to rancidity development and if it is to be kept for any period it must be refrigerated. The addition of an antioxidant such as gum guaiac increases the stability markedly.[26]

Rendered Pork Fat.—This is defined by the Bureau of Animal Industry as follows:[25] "The fats, other than lard, rendered from clean, sound carcasses, parts of carcasses, or edible organs from hogs in good health at the time of slaughter, except that stomachs, tails, bones from the head and bones from the cured or cooked pork are not included. The tissues are usually rendered fresh, but may be cured, cooked, or otherwise prepared and may contain some meat food products. Rendered pork fat may be hardened by the use of lard stearin and/or hardened lard and/or rendered pork for stearin and/or hardened rendered pork fat."

The properties of rendered pork fat are similar to those of lard.

Oleo Stock.—This is the rendered fat from certain selected parts of cattle and calves. It is a harder fat than lard and is used principally to make oleo oil and oleostearine.

Oleo Oil.—Oleo oil is the liquid portion of oleo stock and is separated from the latter by holding it in seeding trucks at 29–32° C. The oleostearine crystallizes and is removed in a hydraulic press. Oleo oil is a bright yellow and has a titer of about 41.5° C. It has good flavor and keeping quality. It is used mainly in margarine and confectionery manufacture.

Oleostearine.—Oleostearine is the residue from oleo oil manufacture. Its titer is about 50° C. It is blended with vegetable oils to make shortenings and is used in candy manufacture.

Beef Tallow.—Beef tallow is made from beef fats not used for oleo stock. Beef tallow has a titer slightly lower than oleo stock.

Mutton Tallow.—This is the rendered fat of sheep. It is much harder than beef tallow, but less stable to oxidation. Its chief use is in shortening.

3. Gelatin

Gelatin is hydrolyzed collagen. It is, therefore, made from certain tissues containing this protein, namely, skin and bones. There are two classes of gelatin, depending upon the material from which they are made: hide gelatin and bone gelatin. Hide gelatin may be made from calf skins or pork skins. Bone gelatin is made from bones of cattle. Variations in properties, other than those due to the source material, are introduced by methods of manufacture.

The important characteristics of a gelatin are color, ability to form a clear water solution, ability to form a gel at definite concentrations and temperatures, the viscosity of a water solution at definite concentrations and temperature, odor, *p*H, bacterial count, and certain specialized functional properties such as quickness of gel formation, ability to form a stiff stable foam or whip (marshmallow manufacture), etc.

---

[26] D. P. Grettie and R. C. Newton, U. S. Patent 1,903,126.

The food uses of gelatin include gelatin desserts, use as a "body" and texture improvers in ice cream, use as an emulsion constituent in many foods, candy making, capsules, pill coating, etc.

4. Casings

Casings are the principal walls of the intestines. Parts of the intestines of all meat animals are made into casings. As manufactured, the casing is largely protein material, since most of the naturally adhering fat is removed. Casings are employed as the containers for comminuted meats of many kinds.

Many varieties depending upon size, shape, and tenderness are used in sausage manufacture. In some instances the casing is not ordinarily eaten; in others, such as with pork sausage or frankfurts it usually is. Where the casing is consumed, tenderness is a desirable feature. Sheep casings are used for pork sausage and frankfurts, as are pork casings. The latter are also used for certain dry sausages. Beef casings are used for both fresh and dry sausage.

Casings are graded as to kind, length, and diameter.

A novel application of enzymes has been made in the tenderization of casings by the use of raw pineapple juice. Bromelin, a proteolytic enzyme, is present in the juice of this fruit. By treatment of the casings of sausages such as pork or frankfurts with fresh pineapple juice, any degree of tenderization of the casing can be accomplished. The tenderizing action can be stopped by application of a sufficiently high temperature to destroy the enzyme.

5. Pharmaceuticals

There exist in the glands of the meat animal substances, usually in comparatively minute quantities, which can be utilized in the treatment of human ailments. Such specialized materials are known as pharmaceuticals. Here we can do no more than to mention the chief pharmaceutical preparations obtained as by-products of the meat-packing industry:

Adrenaline, anterior pituitary gland substance, bile salts, bone marrow folestrin, gastric mucin, histidine, hydrocholine, liver preparations, ovarian preparations, pancreas preparations, pepsin, peptone, pineal substance, posterior pituitary, spleen preparations, suprarenal cortex preparations, thymus, and thyroid.

While not a pharmaceutical, a material of related source, rennin, a stomach enzyme, is used for milk curdling in cheese making.

6. Animal Feeds and Pet Foods

To a great extent, the residue of the carcasses after all the edible and valuable inedible products and by-products have been obtained, go to make up in whole or in part animal feeds. Certain of these animal feeds constitute only a part of the diet of the animals for which they are prepared and are strictly supplementary to the other constituents. Others, such as pet or dog food, may be blended at the meat-packing plant with other materials and used as a complete diet.

(*a*) *Animal Feeds*

There are four basic types of animal feeds: (*1*) wet-rendered tankage, (*2*) dry-rendered tankage, (*3*) blood meal, and (*4*) bone meal. These basic materials are combined in various ways to make up the commercially traded animal feeds.

Wet-rendered tankage is a ground meaty substance, brown in color and smelling like cooked meat. It is the residue from animal tissues which have been cooked or "tanked" under steam and from which the fat has largely been removed. It is generally dried to under 8% moisture. Its protein content is 50 to 60%. Fat runs 8 to 14%.

Dry-rendered tankage is mainly the residue from the rendering of fatty tissue with the addition of some beef. It is considered superior to wet-rendered tankage, although its composition by analysis is similar.

Blood meal is ground dried blood. It is the highest protein (80 to 85%) feed available, and is considered excellent as a supplement for animal feeds.

Bone meal is cooked and ground bone. It is principally calcium phosphate. It is ground to various degrees of fineness depending upon the purpose, *e. g.*, for poultry feed, it may be ground only to the size of a kernel of corn.

(*b*) *Pet Foods*

Canned dog food first made its appearance in the early twenties and has grown to be a major item in the meat-packing business. Meat and meat products (hearts, kidneys, brains, lungs, melts, etc.), cereals, such as barley and wheat, are combined with mineral and vitamin supplements to make a complete diet for pets. A typical analysis of such a dog food is as follows: moisture, 70.5%; fat, 4.4%; protein, 11.2%; ash, 2.8%; carbohydrates, 11.1%.

In early 1942 the use of tin cans was prohibited for dog food in order to conserve tin during the shortage caused by World War II. A few meat packers perfected a dehydrating process that enabled them to manufacture their product with a 6 to 8% moisture content. This low-moisture product can be packed in paper containers. The addition of water to this dehydrated dog food yields a product having about the same physical and nutritional characteristics as those possessed by the canned product.

XVIII. INEDIBLE BY-PRODUCTS

1. Inedible Fat Products

(*a*) *Soap and Glycerol*

The treatment of glyceride fat or a fat acid with an alkali such as sodium hydroxide results in the formation of a soap. Glycerol is also formed if the material treated was a glyceride. This process is known as saponification. Practically all commercial soaps of today are sodium salts of fat acids. Glycerol is a hygroscopic liquid with a sweet taste. Although largely used for such purposes as nitroglycerin manufacture, as a plasticizing agent in cellophane and other materials, as an antidrying agent in tobacco, for the manufacture of glyptal resins, etc., glycerol may also be made in grades which permit its use in such edible products as pharmaceuticals.

While fats from sources other than meat-packing plants are utilized in soap manufacture, such as cocoanut oil and fish oils, meat fats, particularly tallow, represent a large portion of the raw materials available to the industry. Soap manufacture is a huge industry in itself, and although some meat-packing houses have soap factories associated with them, generally speaking the main relationship of the meat-packing industry to soap making is that of supplying raw materials.

(*b*) *Fat and Oil Preparations*

The inedible grades of fats and oils may be utilized as such in certain industrial applications, or they may be modified slightly for certain requirements.

Grease oil (lard oil) is prepared from hog fat in much the same way as oleo oil is made from beef fat. Its pour point and cold test are much lower than oleo oil. Grease oil is used alone or with petroleum oils for metal cutting, leather and textile treating, wool oiling, etc. Soluble oil, made by mixing petroleum oils and grease oil with suitable emulsifying agents, may be diluted as much as 50 times with water.

Neat's-foot oil is obtained from the shins and feet of cattle. It is used in leather working.

Certain varieties of beef tallow are treated with concentrated sulfuric acid to produce sulfated (sulfonated) tallows, which are used in leather manufacture and as technical emulsifying agents. Insoluble metallic soaps dissolved in petroleum fractions improve lubricating greases. Distilled and fractionated fat acids find uses in many industries.

2. Inedible Protein Products

(*a*) *Animal Glue*

Animal glue is made only from those materials rich in collagen. Bones yield bone glue, whereas hide glue is obtained from hide trimmings, sinews, ears, snouts, lips, tails, etc. Chemically glue is related to gelatin, in that it is a product of collagen hydrolysis. Gelatin, however, is made from edible products under strict sanitary conditions so as to yield a material of high purity. Jelly strength and viscosity are two physical constants of importance to both glue and gelatin.

Hide glue is considered superior to bone glue and is used for many purposes such as an adhesive for wood joints, as a binder for the abrasives in emery wheels, as a sizing for rayons and acetates, etc. Bone glue is used where the strength and resistance of hide glue are not required, as in the gluing of paper boxes and for gummed tape.

Glues have many specialized purposes and are frequently modified for a special use. The addition of chemical liquefiers, *e. g.*, urea, has permitted the use of animal glue without heat. "Iceproof" glues for labeling cold-drink bottles have been prepared, which permit immersion of the bottle in cold water without a loosening of the label but which, when washed with hot water, readily release the label. Since glue can be tanned, in much the same ways as hides, the use of certain protein precipitants permits the formation of an insoluble material. An example of such a use is the treatment of animal glue with formaldehyde in binding ground cork for bottle-cap manufacture.

Glues are also used because of their emulsifying and foaming properties, as for example, in the manufacture of matches. They are used as protective colloids for the stabilization of emulsions such as those of asphalt.

(*b*) *Fertilizer*

At the present time no large tonnage of packing house by-products is marketed as fertilizer. Practically all the materials which formerly were sold as fertilizer are now processed into animal feeds. There remains, however, some production of fertilizer of animal origin.

The feeding laws of a number of the states do not permit the addition of horns, hoofs, and hair to animal feeds. Such materials are converted to nitrogen concentrates for use as commercial fertilizers. Other protein materials not particularly suited for animal feeding are also converted to plant foods. Some bone meals running high in nitrogen are employed as fertilizers.

(*c*) *Hides and Skins*

Hides and skins were among the first by-products of the meat-packing industry and today are still by-products of prime importance. Hides are the outer coverings of large and fully grown animals, including cattle. Skins are the outer coverings of smaller animals including calves, pigs, and sheep. Hides and skins vary greatly in texture and thickness in the various parts of the animal, and are also subject to variation with differences in climate and breed.

The meat-packing plant usually salts or brines hides for shipment to the tannery, where they are converted into leather. Pork skins for gelatin manufacture are usually frozen for transportation to the gelatin factory. A relatively small proportion of pork skins is converted to leather.

(*d*) *Wool*

Most of the wool production is obtained from the shearing of sheep before they are marketed as meat animals. Only about 15% of the total wool production has its origin as a meat-packing plant by-product. This wool is obtained by first painting the pelt on the flesh side with a depilatory and after a suitable period of time, pulling the wool out by hand; hence the name, "pulled wool." After pulling, the wool is dried for shipment to the wool houses. Pulled wool can be used for any purpose to which shorn wool can be put, and for some uses it is preferred.

(*e*) *Blood*

In addition to the use of blood for the manufacture of animal feeds, blood may be used in other ways. Separating the red corpuscles leaves the plasma, which is mainly a solution of blood albumin. Blood albumin is now less important as an adhesive, but still finds use in the binding of materials to metal or lacquered surfaces. It is also used in textile sizing, leather finishing, paper coating, etc.

(*f*) *Strings and Ligatures*

Carefully selected portions of lamb intestines are converted into strings for musical instruments, tennis racquets, etc. Ligatures for surgical use are also made from the same raw material, and are products of special importance since the onset of the second World War.

Selected Bibliography

Edelmann, R., Mohler, J. R., and Eichhorn, A., *Meat Hygiene*. Lea and Febiger, Philadelphia, 1939.

Hinman, R. B., and Harris, R. B., *The Story of Meat*. Swift & Company, Chicago, 1942.

Jensen, L. B., *Microbiology of Meats*. Garrard, Champaign, 1942.

Lowe, B., *Experimental Cookery*. Wiley, New York, 1943.

Sherman, H. C., *Chemistry of Food and Nutrition*. Macmillan, New York, 1941.

Tomhave, W. H., *Meats and Meat Products*. Lippincott, Philadelphia, 1925.

Chapter XIV

FISH, SHELLFISH, AND CRUSTACEA

Maurice E. Stansby

Seattle, Washington

I. DESCRIPTION AND EXTENT OF COMMERCIAL FISHERIES[1]

Most of the fisheries of the world are located in countries in the North Temperate Zone or farther north. This distribution is favored by two factors. First, many countries located in this area such as Norway, Japan, and Great Britain support a much larger population than can be fed from local agricultural enterprise, and in areas where much food has to be imported the fisheries usually are exploited to the

Table 71

Extent of World Fisheries[a]

Country	Year	Fishermen thousands	Fishing craft thousands	Volume of catch, billions of pounds	Value of catch, millions of dollars
Japan	1935	1500	365	6.6	87.0
U. S. A. and Alaska	1936	129	78.6	4.8	92.8
U. S. S. R.	1933	250		2.9	86.0
China	1936			2.8	60.0
Great Britain	1936			2.4	82.1
India, Persia, Burma	1932			2.0	20.0
Norway	1937	115	75	1.7	20.3
Germany	1937	30	20	1.5	40.8
Chosen	1937			1.4	4.5
Canada	1937	70	40.5	1.1	23.2
France	1934	144	25.7	0.7	57.8
Spain	1933			0.7	26.0
South and Central America				0.7	14.5
Netherlands	1937	17.6	3.4	0.6	11.8
Iceland	1936			0.4	7.0
Newfoundland	1933	34		0.4	6.0
Portugal	1935	55	14	0.3	3.6
Poland	1935	1.7	0.8	0.3	1.1
All others	. . .			2.3	99.6
Total	.		. .	33.6	744.1

[a] Data taken from material compiled by Division of Fishery Industries, U. S. Fish and Wildlife Service and published in *Fishing Gazette*, Annual Review Number, **1938**, p. 35.

---

[1] Acknowledgment is made to Mr. John Dassow of the staff of the Fishery Products Laboratory, Ketchikan, Alaska, for assistance in compiling data for this section.

utmost. In the second place, there seems to be no doubt that owing to greater abundance of plankton in the waters, fish tend to be more plentiful in temperate waters.

In Table 71 are given statistics of world fisheries, and in this and succeeding tables such statistics are taken from records of prewar years in order to obtain more representative figures on the average conduct of the fisheries.

Fisheries of the United States are conducted in different ways in different sections of the country.

1. Fisheries of New England States

In this region the most important fishing operations center around the taking of ground fish (*i. e.*, those which feed on or near the bottom), the otter trawl being the principal fishing gear used. This method of fishing lends itself to the taking of rather large catches by a single fishing vessel. Accordingly, fishing craft are of considerable size and a large steam trawler commonly lands up to two hundred thousand pounds or more at a time. Haddock and cod are the main species taken by means of the otter trawl and in recent years increasingly large catches of rosefish have been taken as well. Other important species caught by the trawling fleet include flounders, pollock, and whiting. Altogether the catch of the New England otter trawl fishery amounts to more than four hundred million pounds. This fishery is not at all seasonal for the fish are landed throughout the year. Most of the ground fish caught go into either the fresh or frozen markets, and a large portion are sold as fresh or frozen fillets. Owing to their excellent keeping quality and low cost these fish have a wide marketing distribution and are sold throughout the eastern and middle western States.

Fig 40—Fishing fleet at Gloucester, Mass.

A small portion of the ground fish are taken by means of trawl lines, this method formerly accounting for practically the entire catch of such species but now being largely displaced by the otter trawl. A minor portion of the ground fish are also taken in gill nets.

The only other fishery of considerable magnitude carried on in the New England States is that for mackerel. These fish are largely taken by means of purse seines, although a few are caught in traps and in gill nets. Mackerel fishing extends from April through December, although most of the fish are taken during the summer months. This species is largely marketed fresh.

TABLE 72

FISHERIES OF THE NEW ENGLAND STATES[a]

Species	Average annual catch,[b] millions of pounds	Average annual value,[b] millions of dollars	Price per pound to fishermen, cents	Principal methods of catching	Season of commercial fishery	Principal market
Fish:						
Cod	119.7	2.4	2.0	Otter trawl	Year around	Fillets fresh and frozen; salted
Cusk	8.1	0.15	1.8	Lines; otter trawl	Year around	Fresh
Flounder	44.9	1.5	3.4	Otter trawl	Year around	Fresh and frozen; fillets
Haddock	170.0	3.9	2.3	Otter trawl	Year around	Fillets fresh and frozen
Hake	23.8	0.35	1.5	Lines; otter trawl	Year around	Fresh; salted
Halibut	2.2	0.22	10.0	Lines; otter trawl	Year around	Fresh
Herring, sea	51.1	0.35	0.7	Traps	Year around	Canned; smoked
Mackerel	38.2	1.0	2.7	Purse seine, traps	May to Dec.	Fresh
Pollock	37.0	0.52	1.4	Otter trawl; gill nets	Year around	Fresh; frozen fillets
Rosefish	54.5	0.74	1.4	Otter trawl	Year around	Fresh and frozen fillets
Scup, or porgy	9.1	0.22	2.5	Otter trawl	Year around	Fresh
Sea bass	3.2	0.14	4.3	Otter trawl	Year around	Fresh
Swordfish	2.2	0.35	15.6	Harpoon	July to Oct.	Fresh
Whiting	23.3	0.24	1.0	Otter trawl; traps	May to Oct.	Fresh and frozen
Shellfish:						
Crabs, hard	2.6	0.24	9.2	Pots	Year around	Fresh
Lobster	11.1	2.2	19.7	Pots	Year around	Fresh
Clams	17.9	1.4	7.8	Dredges, hoes	Mar. to Nov.	Fresh
Oysters	9.5	1.4	14.7	Dredges	Sept. to May	Fresh
Scallops	6.3	0.91	14.5	Dredges	Year around	Fresh

[a] Includes fish landed in Maine, Massachusetts, Rhode Island, and Connecticut.

[b] Statistics are average for the years 1935 to 1939, inclusive.

The bulk of the New England fish are landed at Boston, Gloucester, New Bedford, Mass., and Portland, Me. The Boston fish pier is the largest landing place for fish in the United States, about three hundred million pounds being handled there annually.

Principal shellfish taken in this region include lobster, crabs, clams, and oysters. The last named are obtained mostly off the coast of Connecticut and Rhode Island, a majority of the clams from Massachusetts, and the lobster and crabs from Maine and Massachusetts. Table 72 gives information concerning the fisheries of the New England States. Statistics in Tables 72 to 78, inclusive, are from the annual reports of Fiedler.[1a]

The principal fishery product manufactured in the New England States is fish fillets. Other products include canned herring (sold as Maine sardines) and salt cod.

2. Fisheries of the Middle Atlantic States

Information concerning fisheries of the Middle Atlantic States (New York, Pennsylvania, New Jersey, and Delaware) is given in Table 73. The fisheries in this region so closely resemble those of adjacent regions that few additional details are necessary. The largest fishing port is New York City, and fishing vessels operating from there closely resemble those of New England, with the otter trawl predominating. The fisheries of New Jersey and the other Middle Atlantic States are similar to those farther south, and the pound net or trap is the principal fishing method for food fish. Oysters and clams are the principal shellfish.

TABLE 73

FISHERIES OF THE MIDDLE ATLANTIC STATES[a]

Species	Average annual catch,[b] millions of pounds	Average annual value,[b] millions of dollars	Price per pound to fishermen, cents	Principal methods of catching	Season of commercial fishery	Principal market
Fish:						
Bluefish	1.9	0.14	7.7	Lines; pound nets; gill nets	Year around	Fresh
Butterfish	7.8	0.28	3.6	Pound nets; otter trawl	Year around	Fresh
Cod	6.7	0.27	3.9	Otter trawl	Year around	Fresh
Flounder	12.3	0.66	5.4	Otter trawl	Year around	Fresh; frozen
Haddock	7.0	0.26	3.7	Otter trawl	Year around	Fresh fillets; frozen
Mackerel	3.6	0.13	3.7	Pound nets	April to Sept.	Fresh
Menhaden	140.9	0.54	0.4	Purse seines; pound nets	April to Dec.	Oil and meal
Scup	7.4	0.15	2.0	Otter trawl; pound nets; purse seines	Year around	Fresh
Sea bass	2.4	0.13	5.4	Pots; otter trawl	Dec. to May	Fresh
Shad	3.4	0.27	8.1	Gill nets; pound nets	April to June	Fresh
Squeteague (gray sea trout)	9.5	0.30	3.1	Pound nets	April to Nov.	Fresh
Whiting	10.1	0.13	1.3	Pound nets; otter trawl	Year around	Fresh
Shellfish:						
Clams, hard	4.9	0.77	...	Tongs; dredges	March to Nov.	Fresh
Oysters	14.3	2.1	...	Dredges; tongs	Sept. to May	Fresh
Scallops, sea	2.8	0.35	...	Dredges	Year around	Fresh

[a] Includes fish landed in New York, New Jersey, and Delaware.
[b] Statistics are average for the years 1935 to 1939, inclusive.

[1a] R. H. Fiedler, *Fishery Industries of the United States;* U. S. Fish and Wildlife Service, 1931–1939.

The principal fishery product manufactured in this area is smoked fish. Many species of fish obtained from other regions are smoked in New York City including salmon from the West Coast and Alaska, and fresh-water fish from the Great Lakes.

3. Fisheries of the Chesapeake Bay States

Menhaden is the species caught in greatest quantity in the Chesapeake Bay States (Maryland and Virginia), but this fish is used entirely for manufacture of oil and fish meal and not for human food. Shellfish are the principal fish in value, this region being the most important area for production of oysters in the world. Other important shellfish taken include clams and crabs.

TABLE 74

FISHERIES OF THE CHESAPEAKE BAY STATES[a]

Species	Average annual catch,[b] millions of pounds	Average annual value,[b] millions of dollars	Price per pound to fishermen, cents	Principal methods of catching	Season of commercial fishery	Principal market
Fish:						
Alewives	17.7	0.17	0.9	Pound nets	April and May	Fresh; salted
Croaker	36.3	0.49	1.3	Pound nets; otter trawl; haul seines	Year around	Fresh
Menhaden	126.7	0.54	0.4	Purse seines	April to Dec.	Oil and meal
Shad	3.6	0.34	9.5	Pound nets; gill nets	March to May	Fresh
Squeteague (sea trout)	13.5	0.24	1.8	Pound nets; otter trawl	April to Nov.	Fresh
Striped bass	2.5	0.19	7.9	Pound nets; gill nets; haul seines	Oct. to May	Fresh
Shellfish:						
Crabs, hard	44.2	0.90	2.0	Trotlines; dredges	Year around	Fresh
Crabs, soft	5.0	0.42	8.5	Scrapes; dip nets	May to Dec.	Fresh
Clams, hard	2.2	0.36	16.4	Tongs	Year around	Fresh
Oysters	32.4	2.38	7.3	Tongs; dredges	Sept. to May	Fresh

[a] Includes fish landed in Maryland and Virginia.
[b] Statistics are average for the years 1935 to 1939, inclusive.

Most food fish in the Chesapeake Bay area are taken in traps, close to the shore. The most important species include croaker, alewives, and squeteague (sea trout). Most important manufactured fishery products include shucked oysters, packaged, cooked crab meat, and salted alewives. Information concerning the fisheries of the Chesapeake Bay states is given in Table 74.

4. Fisheries of the South Atlantic and Gulf States

The total landing of fish in the South Atlantic and Gulf States (North Carolina, South Carolina, Georgia, Florida, Alabama, Mississippi, Louisiana, and Texas) often exceeds four hundred million pounds, but a large part of these are menhaden which are rendered for oil and meal so that food fish amount to only about one hundred million pounds. Principal species include mullet, sea trout, croaker, Spanish mackerel, and red snapper. Many of the food fish (except in the red snapper fishery) are taken close to shore by such gear as traps, gill nets, and seines.

The quantity of shellfish taken in these states considerably exceeds that of all other food fishes. Shrimp is the most important species and it is produced largely by the Gulf States, especially Louisiana, although smaller quantities are obtained from all the States of this region. Crabs are second in importance to shrimp and are plentiful in all the South Atlantic and Gulf States. A considerable quantity of oysters is found, also, especially in Louisiana.

TABLE 75

FISHERIES OF THE SOUTH ATLANTIC AND GULF STATES[a]

Species	Average annual catch,[b] millions of pounds	Average annual value,[b] millions of dollars	Price per pound to fishermen, cents	Principal methods of catching	Season of commercial fishery	Principal market
Fish:						
Bluefish	5.4	0.27	5.0	Gill nets; haul seines	Dec. to April	Fresh
Catfish, bullheads	5.4	0.21	3.9	Haul seines; lines	Year around	Fresh
Croaker	8.1	0.11	1.3	Haul seines; pound nets; gill nets	May to Oct.	Fresh
Drum	5.1	0.23	4.4	Gill nets	Year around	Fresh
Groupers	5.6	0.18	3.2	Lines	Year around	Fresh
King mackerel	3.7	1.15	3.9	Troll lines	Dec. to April	Fresh
Menhaden	261.8	0.81	0.3	Purse seines	April to Dec.	Oil and meal
Mullet	38.0	1.24	3.3	Gill nets; haul seines	Year around	Fresh
Snapper, red	7.7	0.54	7.0	Lines	Year around	Fresh
Spanish mackerel	7.6	0.34	4.4	Gill nets; haul seines	Oct. to April	Fresh
Squeteague (sea trout)	13.8	0.78	5.6	Pound nets; gill nets	Year around	Fresh
Shellfish:						
Crab, hard	27.4	0.41	...	Trotlines; otter trawl	Year around	Fresh
Shrimp	136.9	4.5	...	Otter trawl	Year around	Canned; fresh
Oysters	24.4	1.4	...	Dredges; tongs	Sept. to May	Canned; fresh

[a] Includes fish landed in North Carolina, South Carolina, Georgia, Florida, Alabama, Mississippi, Louisiana, and Texas.

[b] Statistics are average for the years 1935 to 1939, inclusive.

Principal manufactured fishery products from this region include packaged fresh, frozen, canned, and dried shrimp, packaged fresh shrimp, fresh shucked and canned oysters, menhaden oil and meal, and salted alewives and mullet.

Information concerning the fisheries of this region is given in Table 75.

5. Fisheries of the Pacific Coast States

The most important species taken in California, Oregon, and Washington include the various species of salmon and tuna, mackerel, pilchard, and halibut. All these species, except halibut, are utilized largely as canned fish, so that the fisheries of the West Coast center around the fish canneries which are located at San Diego, Terminal

Island (near Los Angeles), and Monterey in California, and Astoria, Ore., at the mouth of the Columbia River. Other important fishing centers include San Francisco and Seattle, the latter being the principal port for landing halibut in the United States. Wide varieties of fishing methods are used in this section. Data on the fisheries of the Pacific Coast States are given in Table 76.

TABLE 76

FISHERIES OF THE PACIFIC COAST STATES[a]

Species	Average annual catch,[b] millions of pounds	Average annual value,[b] millions of dollars	Price per pound to fishermen, cents	Principal methods of catching	Season of commercial fishery	Principal market
Fish:						
Barracuda	3.0	0.15	5.0	Purse seines; gill nets	Year around	Fresh
Cod	12.1	0.18	1.4	Lines	Aug. to May	Fresh; salted
Flounder	16.5	0.72	4.4	Otter trawls	Year around	Fresh; frozen
Halibut	24.8	2.1	8.4	Lines	April to Sept.	Fresh; frozen
Mackerel	93.7	0.89	1.0	Lampara nets; purse seines	June to Mar.	Canned
Pilchard	1232.0	6.3	0.5	Purse seines; lampara nets	Aug. to Mar.	Oil and meal; canned
Rockfish	4.9	0.19	3.9	Lines	Year around	Fresh
Sablefish	4.4	0.18	4.1	Lines	June to Nov.	Smoked; frozen
Salmon blueback or red	6.2	0.56	9.1	Gill nets; troll lines	July and Aug.	Canned
Salmon chinook or king	29.0	2.4	8.3	Gill nets; troll lines	April to Sept.	Canned; mild cured
Salmon chum or keta	10.5	0.24	2.3	Purse seines	Oct. and Nov.	Canned
Salmon pink or humpback	15.0	0.37	2.5	Purse seines	July to Sept.	Canned
Salmon silver or coho	19.0	0.98	5.2	Troll lines; gill nets	June to Oct.	Canned
Sharks[c]	9.8	0.17	1.8	Lines; gill nets	Sept. to Mar.	Fresh
Smelt	3.8	0.13	3.3	Purse seines; gill nets	Year around	Fresh
Tuna albacore	8.7	0.51	5.9	Lines; purse seines	July to Oct.	Canned
Tuna bluefin	17.3	0.86	5.0	Lines; purse seines	May to Sept.	Canned
Tuna bonita	8.1	0.27	3.3	Lines; purse seines	Feb. to Nov.	Canned
Tuna shipjack	28.8	1.3	4.5	Lines; purse seines	Year around	Canned
Tuna yellowfin	86.2	4.8	5.6	Lines; purse seines	Year around	Canned
Yellowtail	6.7	0.22	3.3	Lines: purse seines	June to Oct.	Canned
Shellfish:						
Crabs, hard	10.1	0.67	6.6	Pots; hoop nets	Feb. to June	Canned; fresh
Lobster, spiny	1.3	0.18	13.9	Pots; traps	Sept. to April	Fresh
Clams, razor	0.92	0.15	16.3	Digging	March to May	Canned
Oysters, Pacific	7.5	0.51	6.8	Rakes	Nov. to May	Fresh; canned

[a] Includes fish landed in Washington, Oregon, and California.

[b] Statistics are average for the years 1935 to 1939, inclusive.

[c] Statistics are average for 1937, 1938, and 1939 only.

The principal manufactured fishery products of this region are the canned fish—salmon, tuna, pilchard (sardines), mackerel, and oysters—and the manufacture of pilchard meal and oil. The most important shellfish taken in these states are crabs and oysters.

6. Fisheries of Alaska

As shown in Table 77, the fisheries of Alaska are limited largely to the various species of salmon, halibut, and herring. Fishing operations in Alaska are very seasonal, since all these species are caught only during the summer months. In some regions the

TABLE 77
FISHERIES OF ALASKA

Species	Average annual catch,[a] millions of pounds	Average annual value,[a] millions of dollars	Price per pound to fishermen, cents	Principal methods of catching	Season of commercial fishery	Principal market
Fish:						
Halibut	17.6	0.90	5.1	Lines	April to Sept.	Frozen; fresh
Herring	186.8	0.93	0.5	Traps; seines	June to Oct.	Oil and meal; salted
Salmon, red	180.1	4.48	2.5	Gill nets; traps	June and July	Canned
Salmon, king	18.1	0.45	2.5	Troll lines	Year around	Mild cured; canned
Salmon, chum	82.2	0.86	1.0	Purse seines; traps	Aug. to Oct.	Canned
Salmon, pink	260.2	3.81	1.5	Traps; purse seines	July and Aug.	Canned
Salmon, silver	18.6	0.39	2.1	Traps; purse seines; gill nets	April to Sept.	Canned
Shellfish:						
Crabs, hard	0.94	0.085	9.0	Pots	Sept. to May	Canned
Shrimp	0.80	0.032	4.0	Beam trawls	May to Jan.	Cold packed
Clams, razor	0.82	0.042	5.1	Digging	Nov. to June	Canned

[a] Statistics are average for the years 1935 to 1939, inclusive.

TABLE 78
FISHERIES OF INLAND LAKES AND RIVERS[a]

Species	Average annual catch,[b] millions of pounds	Average annual value,[b] millions of dollars	Price per pound to fishermen, cents	Principal methods of catching	Season of commercial fishery	Principal market
Lakes:						
Blue pike	12.2	0.79	6.4	Trap nets; gill nets	May to Nov.	Fresh; fillets
Carp	5.2	0.16	3.0	Haul seines	Year around	Fresh
Chubs	5.7	0.77	13.6	Gill nets	Year around	Smoked; fresh
Herring, lake	21.4	0.62	2.9	Gill nets	Year around	Fresh
Lake trout	9.5	1.6	16.6	Gill nets	Year around	Fresh
Sauger	1.6	0.12	7.4	Trap nets; gill nets	Sept. to April	Fresh
Suckers	5.4	0.15	2.9	Trap nets; pound nets	Year around	Fresh
Whitefish	3.7	0.70	19.2	Gill nets; trap nets; pound nets	Year around	Smoked
Yellow perch	6.1	0.48	7.8	Trap nets; gill nets	Year around	Fresh
Yellow pike	5.6	0.55	9.8	Trap nets	Year around	Fresh
Rivers:						
Buffalo fish	15.8	0.69	4.4	Fyke nets; haul seines	Year around	Fresh
Carp	11.9	0.46	3.8	Haul seines; fyke nets; gill nets	Year around	Fresh
Catfish, etc.	10.3	0.88	8.5	Fyke nets	Year around	Fresh
Sheepshead	3.9	0.14	3.7	Fyke nets	Year around	Fresh

[a] Includes fish landed from lakes in New York, Pennsylvania, Ohio, Michigan, Indiana, Illinois, Wisconsin, and Minnesota, and from the Mississippi River and its tributaries.

[b] Statistics for lakes are for the years 1936 to 1939, inclusive; for rivers the statistics are for the year 1931, which is the latest available.

season lasts for only 6 weeks to 2 months. Canned salmon is by far the most important manufactured fishing product put up in Alaska. Small quantities of crabs, razor clams, and shrimp are also canned. Much of the halibut caught off Alaskan waters is landed in Prince Rupert, B. C., or Seattle, and does not appear in Table 77.

7. Fisheries of Inland Lakes and Rivers

More than one hundred million pounds of fish are taken annually from inland lakes and rivers, the largest portion coming from the Great Lakes. Information concerning these fisheries is given in Table 78.

II. COMPOSITION OF FISH[2]

1. Variation in Composition

Before considering the chemical composition of fish it should be realized that there is a considerable variation in such composition, so that analyses made on only a few samples cannot be considered representative and, in fact, in many cases variation is so great that average composition is of only theoretical interest since actually individual fish will vary so widely from such average values.

TABLE 79

SEASONAL VARIATION OF OIL CONTENT OF MACKEREL (*Scomber scombrus*)[a]

Date fish were caught	Number of fish analyzed	Maximum oil content, %	Minimum oil content, %	Average oil content, %
April 18	13[b]	..	..	3.9
April 22	8	7.6	2.7	4.8
May 3	4[b]	..	..	8.0
May 21	4[b]	..	..	9.0
June 1	6[b]	..	..	9.8
July 23	13	17.3	6.1	10.6
Aug. 13	7	25.6	10.6	17.5
Aug. 15	7	21.6	16.0	19.2
Sept. 11	20	11.4	2.0	6.5
Oct. 1	25	16.2	3.0	10.1
Nov. 17	12	15.2	2.2	8.2

[a] M. E. Stansby and J. M. Lemon, U. S. Fish and Wildlife Service, *Investigational Rept.* 1 (1941).
[b] Composite samples.

Doubtlessly the component of fish varying to the greatest degree is the oil (or fat) content. Many species of fish store oil when feeding as a reserve to draw upon later. Such fish generally migrate over vast distances and during the migration may not feed upon anything except their stored oil reserve. This causes a seasonal variation in oil content as shown in Table 79. The oil content of mackerel may vary by as much as eleven times, and even in a single lot of fish caught together it may vary by as much as seven times. In addition to season of the year, other factors which may cause variation of oil content of fish include nature of food of fish, locality where fish are caught, and size and age of mackerel. The smaller fish show less variation of oil content than do the large fish (Table 80).

---

[2] Acknowledgment is made to Mr. Frank Piskur of the staff of the Fishery Products Laboratory, Ketchikan, Alaska, for assistance in compiling data for this section.

TABLE 80

EFFECT OF SIZE OF MACKEREL ON OIL CONTENT[a]

Length of fish, cm.	Probable age group, years	Average oil content, %		
		June 5	Oct. 20	Nov. 17
30 -31.5	1	...	14.6	9.4
31.5-36.5	2	9.3	13.6	22.0
36.5-38	3	7.0	16.5	20.1
39 -42	5 and 6	4.0	17.1	16.5
41 -44	7 and over	2.2	...	...

[a] M. E. Stansby and J. M. Lemon, U. S. Fish and Wildlife Service, *Investigational Rept.* 1 (1941).

While the oil content of fish varies more widely than other components most of the other substances present show some variation. The water content of many species varies inversely with the oil content such that on the edible portions of the flesh, the sum of the water and oil content is usually quite close to a constant value.

Fish also vary in composition at different sections of the same fish as shown in Table 81. The oil content is generally lower near the tail than near the head. This is an important consideration for purchasers of fish, since those desiring a lean fish should procure tail cuts while those preferring fish to be quite oily can specify cuts from near the head.

TABLE 81

VARIATION IN COMPOSITION OF DIFFERENT PORTIONS OF THE SAME FISH[a]

Species	Cross section analyzed	Fat, %	Protein, %	Ash, %
Salmon (red chinook)	Near head	20.2	17.6	1.19
Salmon (red chinook)	Near tail	11.1	17.9	1.12
Salmon (white chinook)	Near head	15.1	19.0	1.40
Salmon (white chinook)	Near tail	8.1	19.9	1.31
Yellowtail	Middle	3.21	19.8	1.35
Yellowtail	Near tail	1.38	21.0	1.35
Skipjack	Belly only	25.8	18.3	1.35
Skipjack	White meat only	15.4	21.9	1.39
Skipjack	Dark meat only	22.4	18.2	1.03

[a] H. Beard (editor), *Rept. U. S. Commissioner of Fisheries for 1925* (1926), Appendix 10, p. 501.

2. Gross Composition

Tables of average gross composition of fish are apt to be misleading as stated in the previous section but if their limitations are realized they may be useful to obtain an approximation of the composition of the various species.

No comprehensive analyses of gross composition of fish have been made since the very exhaustive studies of Atwater.[2a] Although these analyses were made more than 50 years ago the methods of analysis are beyond reproach. Unfortunately proper consideration was not given to seasonal variation in composition which greatly limits the value of this report. Table 82 summarizes the best available data on oil and protein content of fish. In most instances it has been necessary to use data of Atwater, but whenever more recent, reliable data have been available, they have been included. The older tables generally give analyses on other components such as ash, water, and various nitrogen fractions such as coagulable, hot water soluble, etc. The ash content averages about 1.3% for all species. It is reasonably constant between about 1.0 and 1.5% and its variation has no especial significance. Inasmuch as the water content has no nutritional significance, its inclusion likewise seems unnecessary.

TABLE 82

OIL AND PROTEIN CONTENT OF THE EDIBLE PORTION OF FISH

Species	Average oil content, %	Protein content, %
Fish:		
Alewives	5	19
Cod	0.4	17
Croaker	3	18
Flounder	0.6	16
Haddock	0.3	18
Halibut	5	19
Herring, sea	11	19
Mackerel, common	13	18
Mullet	5	19
Pollock	0.8	20
Salmon, king[a]	16	18
Salmon, sockeye[a]	11	21
Salmon, coho[a]	8	21
Salmon, pink[a]	6	21
Salmon, chum[a]	5	21
Sardine (pilchard)	13	23
Squeteague (sea trout)	2	19
Tuna, skipjack[a]	4	22
Whiting	0.4	17
Shellfish:		
Clams	1	9
Crabs	2	17
Oysters	1	7
Shrimp	1	25

[a] Canned.

3. Protein Content

The mere statement of protein content of fish (generally computed by multiplying the nitrogen content by a factor) as given in Table 82 gives very little information as to the relative nutritive value of the fish. Thus in the dogfish, much of the nitrogen occurs as urea so that the protein content, calculated from nitrogen analyses, is quite erroneous. Even assuming the protein content to be accurate, as determined, a more precise knowledge of the composition of the protein is necessary before any decision

---

[2a] W. O. Atwater, *U. S. Fish Commissioner's Rept.*, 1888, p. 679.

TABLE 83

PERCENTAGE OF CERTAIN ESSENTIAL AMINO ACIDS IN FISH AND SHELLFISH[a]

Species	Scientific name	Date sample was prepared	Arginine	Histidine	Lysine	Tryptophane	Cystine
Fish:							
Catfish	*Ameiurus catus*	Aug., 1937	..	..	..	0.97	..
Cod	*Gadus callarias*	June, 1936	5.58	1.72	6.83	1.06	1.41
Croaker	*Micropogon undulatus*	May, 1936	5.81	1.37	6.10	1.24	1.15
Haddock	*Melanogrammus aeglefinus*	Sept., 1935	5.70	1.17	6.41	0.85	1.16
Halibut	*Hippoglossus hippoglossus*	Dec., 1936	6.00	1.66	6.16	1.64	1.45
Herring:							
Lake	*Leucichthys artedi*	Nov., 1937	..	..	..	1.25	..
Sea	*Clupea harengus*	March, 1937	5.09	1.56	7.03	1.23	..
Lake trout	*Cristivomer namaycush*	June, 1937	5.73	1.40	7.15	1.17	..
Mackerel:							
Boston	*Scomber scombrus*	Aug., 1936	5.78	1.93	7.13	1.36	1.18
Spanish	*Scomberomorus maculatus*	Jan., 1936	5.27	1.48	6.53	1.37	1.25
Mullet	*Mugil* species	Feb., 1937	5.78	1.61	6.74	1.36	1.29
Pilchard	*Sardina caerulea*	March, 1937	5.60	1.23	6.78	1.30	..
Red snapper	*Lutianus blackfordii*	Nov., 1936	6.18	1.57	6.72	1.22	1.29
Salmon:							
Chum	*Oncorhynchus keta*	May, 1938	5.55	1.30	5.69	1.33	..
King	*Oncorhynchus tschawytscha*	April, 1937	5.02	1.41	6.27	1.20	1.27
Pink	*Oncorhynchus gorbuscha*	April, 1937	..	..	..	1.09	1.15
Silver	*Oncorhynchus kisutch*	Jan., 1937	5.68	1.87	6.57	1.44	1.39
Sockeye	*Oncorhynchus nerka*	Sept., 1937	..	..	..	1.25	..
Shad	*Alosa sapidissima*	May, 1936	4.54	1.09	6.45	1.22	1.17
Squeteague (sea trout)	*Cynoscion regalis*	June, 1937	5.90	1.42	6.78	1.01	..
Tuna:							
Albacore	*Germo alalunga*	Nov., 1937	..	..	..	1.18	..
Bluefin	*Thunnus saliens*	Oct., 1937	..	..	..	1.25	..
Bonito	*Sarda chilensis*	Sept., 1937	..	..	..	1.19	..
Skipjack	*Euthynnus pelayms*	Dec., 1937	..	..	..	1.16	..
Shellfish and crustacea:							
Clam, hard	*Venus mercenaria*	Sept., 1936	5.27	1.45	5.40	1.19	..
Crab, blue	*Callinectes sapidus*	Nov., 1936	7.61	1.51	6.38	1.11	..
Oyster	*Ostrea virginica*	Oct., 1936	5.71	1.79	5.24	1.67	..
Shrimp	*Peneus brasiliensis*	June, 1936	7.50	1.61	7.35	0.96	1.25
Proteins from other foods:							
Casein	.....	...	5.2	2.6	7.6	2.2	0.3
Beef round	.....	...	7.5	1.8	7.6	0.9	1.3
Egg albumin	.....	...	6.0	2.3	3.8	1.3	0.9

[a] S. R. Pottinger and W. H. Baldwin, *Proc. Pacific Sci. Congr., 6th Congr., Calif.* (1939), p. 453.

as to the relative nutritive value can be made. Table 83 gives analyses for arginine, histidine, lysine, tryptophane, and cystine on certain species of fish. These are five of the amino acids which have been found to be essential to growth. For comparison, analyses on certain other proteins have been included. The analyses of fish and shellfish for these five amino acids compare very favorably with the analyses for casein (principal protein in milk), beef round, and egg albumin.

4. Mineral Content

Newell and McCollum[3] made a semiquantitative spectrographic analysis of certain fish meals and found relatively large amounts of calcium, copper, iron, magnesium, phosphorus, potassium, sodium, and strontium to be present. Smaller amounts of aluminum, fluorine, lithium, and manganese were present, and small traces of barium, chromium, lead, silicon, titanium, vanadium, and zinc were found in most samples. A few samples were found to contain small traces of boron, nickel, niobium, silver, and tin. While some of these elements may represent contaminants picked up during manufacture of the fish meal, it appears probable that most of them were present initially in the fish from which the meal was prepared. In addition to these elements chlorine and sulfur are always found in relatively large quantities, as well as smaller traces of bromine and iodine.

Table 84 gives analyses for certain mineral constituents of nutritional importance in a number of species. Of the elements listed, iodine, although occurring in the smallest quantities, is the most important. This element, so essential in the prevention of goiter, is more abundant in sea foods than in any other natural foodstuff. Oysters are also a very excellent source of iron and copper, being exceeded in content of these minerals only by pork liver and beef liver. Canned salmon, owing to the presence of the softened, edible bone, is an important source of calcium, being exceeded only by cheese and certain nuts in this respect. Other canned fish in which the bone is not removed are equally good sources of calcium.

Fish contain considerably higher quantities of fluorine than do most foodstuffs, averaging about 5 p. p. m. according to Lee and Nilson.[4] This compares with 0.5 to 2.0 p. p. m. of fluorine in most other common foods. Since fluorine in excess of 2 p. p. m. in drinking water will cause mottled teeth, and since the U. S. Food and Drug Administration prohibits *added* fluorine in foods in excess of 1.4 p. p. m., it might appear that the presence of 5 p. p. m. in sea foods would have a harmful effect. Lee and Nilson[4] have shown that this is not the case, however. Apparently the fluorine is present in some form such that assimilation is much less than is the case with added inorganic fluorides. Similarly, Coulson, Remington, and Lynch[5] have shown that the relatively high copper content of oysters has no harmful effect, and that there is no rapid accumulation of copper in the body when large quantities of oysters are consumed.

Arsenic has also been reported[6] to occur in sea foods in quantities higher than in most other foods. Analyses showed arsenic to be present in various species expressed as parts per million as follows: British oysters, 5; Portuguese oysters, 50; mussels, 82; lobster, 37; shrimp, 24; crabs, 46; crawfish, 32; sole, 7. Coulson,

---

[3] J. M. Newell and E. V. McCollum, U. S. Bur. Fisheries, *Investigational Rept.* 5 (1931).

[4] Chas. F. Lee and H. W. Nilson, U. S. Bur. Fisheries, *Investigational Rept.* 44 (1939).

[5] E. J. Coulson, R. E. Remington, and K. M. Lynch, U. S. Bur. Fisheries, *Investigational Rept.*, 23 (1934).

[6] A. C. Chapman, *Analyst*, **51**, 548 (1926).

TABLE 84

THE MINERAL CONTENT OF THE EDIBLE PORTION OF CERTAIN FISH[a]

Species	Number of samples	Dry matter, %[b]	Calcium, %	Magnesium, %	Phosphorus, %	Iron, %	Copper, %	Iodine, %
Fish:								
Cod (*Gadus morrhua*)	4	17.7	0.0110	0.0280	0.1859	0.000518	0.000041	0.000103
Haddock (*Melanogrammus aeglefinus*)	4	18.7	0.0165	0.0236	0.1731	0.000516	0.000041	0.000513
Mackerel (*Scomber scombrus*)	2	19.9	0.0048	0.0281	0.2169	0.001224	0.000115	0.000053
Red snapper (*Lutianus blackfordii*)	3	21.7	0.0162	0.0276	0.2279	0.001158	0.000038	0.000031
Mullet (*Mugil cephalus*)	3	23.9	0.0261	0.0318	0.2198	0.001779	0.000082	0.000485
Pilchard, California (*Sardinia caerulea*)[c]	2	20.5	0.0422	0.0237	0.2115	0.002483	0.000166	0.000013
Flounder (*Pleuronectidae species*)	2	21.3	0.0117	0.0305	0.2053	....	....	0.000029
Lake herring (*Leuichthys artedi*)	1	17.9	0.0116	0.0172	0.1518	....	....	....
Salmon, canned, red (*Oncorhynchus nerka*)	3	31.3	0.2082	0.0292	0.3364	0.001180	0.000081	0.000053
Salmon, canned, chinook (*Oncorhynchus tschawytscha*)	3	33.2	0.1071	0.0267	0.2778	0.001270	0.000077	0.000067
Salmon, canned, coho (*Oncorhynchus kisutch*)	2	30.1	0.2304	0.0298	0.3382	0.000890	0.000064	0.000023
Salmon, canned, pink (*Oncorhynchus gorbuscha*)	2	29.6	0.1735	0.0299	0.3206	0.000760	0.000056	0.000021
Salmon, canned,chum (*Oncorhynchus keta*)	2	27.3	0.2492	0.0299	0.3518	0.000740	0.000050	0.000022
Shellfish:								
Oysters, Eastern (*Ostrea virginica*)	4	15.0	0.0579	0.0320	0.1121	0.006100	0.003730	0.000049
Oysters, Pacific natives (*Ostrea lurida*)	2	17.9	0.0632	0.0242	0.1540	0.004940	0.001240	0.000030
Oysters, Pacific, Japanese (*Ostrea gigas*)	2	21.4	0.0628	0.0480	0.1922	0.007510	0.001230	0.000036
Shrimp (*Peneus brasiliensis*)								
Raw	4	20.0	0.0542	0.0421	0.2285	0.002188	0.000331	0.000023
Boiled	2	28.7	0.0614	0.0509	0.2432	0.003973	0.000302	0.000021
Blue crab (*Callinectes sapidus*)								
White meat	4	21.1	0.1028	0.0336	0.2052	0.002262	0.001582	0.000042
Claw meat	3	20.4	0.0706	0.0345	0.1796	0.000746	0.000368	0.000015

---

[a] H. W. Nilson and E. J. Coulson, U. S. Bur. Fisheries, *Investigational Rept.* 41 (1939).

[b] Samples first dried on steam bath and finished in electric air oven at 80° C.

[c] Whole fresh fish.

NOTE: Four units to right of decimal point equals parts per million or mg. per kg.

Remington, and Lynch[7] have shown that the arsenic present in shrimp is organically combined in a form not readily absorbed during metabolism.

5. Composition of Fish Oils

The components of fish oils have been classified[8] as follows:

Group A:
  Fatty acid esters of glycerol
    sterols
    glycerol ethers
    phospholipids
    vitamins
    pigments

Group B:
  Waxes (fatty acid esters of fatty alcohols)
  Pigments
  Dissolved nitrogenous matter
Group C:
  Hydrocarbons (including some pigments)
  Fatty alcohols

The principal component of most fish oils is the first one in this classification, namely, glycerides of fatty acids, and in true fish oils the glycerides usually make up at least 95% of the oil. The fatty acid components consist of straight-chain even-numbered 14 to 24 carbon acids with the carboxyl group at one end. The degree of unsaturation of the fatty acids increases with the number of carbon atoms. This degree of unsaturation is usually not greater than −4H for acids up to 18 carbon atoms but may be as high as −10H or more for the C_{20}, C_{22}, and C_{24} acids (see page 565).

The saturated fatty acids in fish (Teleostomi) oils make up between about 10 and 25% of the total fatty acids present, and the principal acid present is palmitic. Myristic acid is the second most important of the saturated acids, and only traces of stearic acid occur in most species.

Of the unsaturated acids present in Teleostomi fish oils, only traces of C_{14} acids occur. Of the C_{16} acids palmitoleic acid is the principal one with small amounts of C_{16} acids of higher degree of unsaturation. Oleic acid is the principal component of the C_{18} unsaturated acids with smaller amounts of linolenic and moroctic acids present in some cases. C_{20} acids average 22.5% of the total unsaturated acids and C_{22} acids about 13%.

There seems to be little difference in composition between marine liver oils as a class and marine body oils. There is a slightly higher amount of saturated acids in the body oils, but the difference is not pronounced. Composition of the unsaturated acids shows no decided differences for these classes of oils.

Cholesterol is the principal sterol present in fish oils. It occurs in quite large amounts in some species (up to 35% of the oil from the moon fish). Lecithin is the principal phospholipid found in marine oils. It is present in especially high quantities (up to 20%) in oil from fish eggs, and also in various shark and dogfish liver oils. The amount present in most whole fish averages less than 0.5%. Three glycerol ethers, chymyl alcohol (glycerol α-ether of cetyl alcohol), batyl alcohol (glycerol α-ether of octadecyl alcohol), and selachyl alcohol (glycerol α-ether of

---

[7] E. J. Coulson, R. E. Remington, and K. M. Lynch, *J. Nutrition*, **10**, 255 (1935).

[8] H. N. Brocklesby (editor), Fishery Research Board of Canada, *Bull.* **59** (1941).

TABLE 85

VITAMIN A AND D CONTENT OF FISH-LIVER OILS[a]

Common name	Scientific name	Area in which fish are caught	Source of oil	Oil content, %	Vitamin A range, I. U. per g. oil	Vitamin A average, I. U. per g. oil	Vitamin D range, I. U. per g. oil	Vitamin D average, I. U. per g. oil
Cod family:								
Cod	*Gadus callarias*	Atlantic	Liver	40–60	1000–6000	2000	50–150	125
Gray cod	*Gadus macrocephalus*	Pacific	Liver	20–40	1000–30,000	12,000	100–500	250
Gray cod	*Gadus macrocephalus*	Pacific	Viscera	1–5	8000–100,000	50,000	10–35	...
Haddock	*Melanogrammus aeglefinus*	Atlantic	Liver	...	400–3000	...	30–75	...
Pollock	*Pollachius virens*	Atlantic	Liver	..	...	...	...	...
Pollock	*Theragra chaleogramma*	Alaska	Liver	30	16,000[b]	...	...	...
Whiting	*Merluccius bilinearis*	Mass.	Liver	...	20,000[b]	...	1000[b]	...
Flatfishes:								
Arrowtooth halibut	*Atheresthes stomias*	Pacific	Liver	10–15	10,000–80,000	...	...	...
Cape sole	*Eopsetta jordani*	Pacific	Liver	10–20	100,000–250,000	...	...	...
Cape sole	*Eopsetta jordani*	Pacific	Viscera	1–5	100,000–500,000	...	...	...
English sole	*Parophrys vetulus*	Pacific	Liver	5–10	<5000	...	...	...
Halibut	*Hypoglossus stenolepis*	Pacific	Liver	10–30	30,000–200,000	100,000	1000–5000	...
Starry flounder	*Platichtys stellatus*	Pacific	Liver	10–15	1000–25,000	...	1000[b]	...
Turbot	*Rheinhartius hippoglossoides*	Atlantic	Liver	...	10,000	...	300–400	...
Yellow sole	*Pseudopleuronectes dignabilis*	...	Liver	..	...	..	90[b]	...
Rockfishes:								
Bocaccio	*Sebastodes paucispinis*	Pacific	Liver	...	5000–50,000	...	300–5000	...
Black rockfish	*Sebastodes mystinus*	...	Liver	..	...	..	1500[b]	...
Red rockfish (cod)	*Sebastodes ruberrimus*	Pacific	Liver	5–10	90,000–500,000	250,000	1000–5000	...
Rosefish	*Sebastes marinus*	Atlantic	Body	..	3600[b]	...	50>[b]	...
Salmon:								
Chum	*Oncorhynchus keta*	Pacific	Liver	2–6	5000–15,000	...	100–500	...
Chum	*Oncorhynchus keta*	Pacific	Cannery waste	5–10	Nil	...	50[b]	...
King	*Oncorhynchus tschawytscha*	Pacific	Liver	4–8	10,000–40,000	25,000	100–500	...
King	*Oncorhynchus tschawytscha*	Pacific	Offal	10–15	1500–2000	1000	50–150	100
Pink	*Oncorhynchus gorbuscha*	Pacific	Liver	4–6	1000–40,000	25,000	100–600	...
Pink	*Oncorhynchus gorbuscha*	Pacific	Offal	10–12	500–3000	1500	100–300	150
Silver	*Oncorhynchus kisutch*	Pacific	Liver	4–6	10,000–30,000	...	100–500	...
Silver	*Oncorhynchus kisutch*	Pacific	Offal	10–15	500–3000	1500	100–200	100
Sockeye	*Oncorhynchus nerka*	Pacific	Liver	5–8	10,000–50,000	..	200–600	...
Sockeye	*Oncorhynchus nerka*	Pacific	Offal	10–20	500–5000	2000	100–300	150
Steelhead	*Salmo gairdneri*	Pacific	Liver	10–20	10,000–20,000	. .	100–500	...

Sharks and skates:								
Big skate	*Raja binoculata*	Pacific	Liver	30–60	500–3000	1000	5–25	...
Basking shark	*Cetorhinus maximus*	Calif.	Liver	70–80	0–1000	...	...	...
Bay shark	*Eulamia lamiella*	Mexico	Liver	50–80	15,000–100,000	50,000	...	...
Unknown	*Eulamia aethalorus*	Mexico	Liver	60–80	3000–15,000	7000	...	...
Unknown	*Eulamia azureus*	Mexico	Liver	68[b]	15,000[b]	...	...	...
Unknown	*Eulamia* sp. (*pilota*)	Mexico	Liver	30–60	7000–100,000	60,000	...	...
Unknown	*Eulamia velox*	Mexico	Liver	90–90	16,000–25,000	21,000	...	...
Dogfish or grayfish	*Squalus suckleyi*	Pacific	Liver	50–80	500–100,000	14,000	5–25	...
Dogfish or grayfish	*Squalus suckleyi*	Wash.	Body	10–15	0–1000	...	5–25	...
Dogfish	*Squalus acanthias*	Atlantic	Liver	40–60	<2000	...	...	...
Gray smooth hound shark	*Mustelus Californicus*	Calif.	Liver	40–60	2000–20,000	...	...	...
Hammerhead shark	*Sphyrna zygaena*	Mexico	Liver	50–70	20,000–100,000	40,000	...	...
Leopard shark	*Triakis semifasciata*	...	Liver	...	500–4000	...	...	...
Long nose shark	*Scoliodon longuris*	Mexico	Liver	<70	43,000[b]	...	...	...
Nurse or sleeper shark	*Somniosus microcephalus*	...	Liver	...	400[b]	...	...	...
Ratfish	*Hydrolagus colliei*	Pacific	Liver	70–85	100–1000	...	0–5	...
Soupfin shark	*Galeorhinus zyopterus*	Pacific	Liver	40–70	20,000–500,000	100,000	5–25	...
Starry skate	*Raja stellulata*	Alaska	Liver	10–30	4000–30,000	12,000	...	...
Sand shark	*Eulamia taurus*	Fla.	Liver	...	30,800[b]	...	...	...
Mackerel and tuna:								
Albacore	*Germo alalunga*	Pacific	Liver	5–15	30,000>	...	25,000–250,000	...
Bluefin tuna	*Thunnus thynnus*	All seas	Liver	5–15	20,000–300,000	80,000	20,000–70,000	50,000
Bluefin tuna	*Thunnus thynnus*	All seas	Offal	...	100–1000	...	75[b]	...
Bonito	*Gymnosarda pelamis*	S. America	Liver	...	14,000[b]	...	20,000[b]	...
Bonito	*Sarda chilensis*	Calif.	Liver	...	120,000[b]	...	50,000[b]	...
Striped tuna	*Katsuwonus pelamis*	Pacific	Liver	...	30,000>	...	25,000–250,000	...
Yellowfin tuna	*Neothunnus macropterus*	...	Liver	...	50,000[b]	...	45,000[b]	...
Pacific mackerel	*Pneumatophorus diego*	Calif.	Liver	4–25	15,000–211,000	...	1000–6000	...
Pacific mackerel	*Pneumatophorus diego*	Calif.	Offal	...	1700[b]	...	75[b]	...
Mackerel, common	*Scomber scombrus*	Mass.	Liver	...	500–88,000	...	500>	...
Miscellaneous:								
Black cod	*Anoplopoma fimbria*	Wash.	Liver	10–30	15,000–200,000	50,000	<1000	...
Black cod	*Anoplopoma fimbria*	Wash.	Viscera	5–15	10,000–100,000	...	...	...
Black sea bass	*Stereolepsis gigas*	Pacific	Liver	10–30	200,000–1,000,000	...	5000>	...
Bullhead	*Myoxocephalus* species	Alaska	Liver	5–20	3000–40,000	...	...	...
Barracuda	*Sphyraena argentea*	...	Liver	...	40,000[b]	...	2000[b]	...
Conger eel	*Leptocephalus conger*	...	Body	...	1500[b]	...	200[b]	...
Herring	*Culpea pallasii*	Pacific	Body	5–25	50–300	90	20–80	...
Lamprey eel	*Entosphenus tridentatus*	Columbia Riv.	Viscera	<50	38,000[b]	...	...	...
Lamprey eel	*Entosphenus tridentatus*	Columbia Riv.	Body	<30	2000[b]	...	...	...
Ling cod	*Ophiodon elongatus*	Pacific	Liver	5–20	50,000–500,000	...	1000–6000	...
Ling cod	*Ophiodon elongatus*	Pacific	Viscera	2–16	20,000–150,000	...	100–200	...
Menhaden	*Brevoorta tyrannus*	Atlantic	Body	5–20	<500	...	<100	...
Pilchard	*Sardinops caerulea*	Pacific	Body	5–25	50–800	100	20–100	80
Swordfish	*Xyphias gladius*	Atlantic	Liver	15–25	20,000–400,000	250,000	2000–25,000	14,000
White sea bass	*Cynoscion nobilis*	Calif.	Liver	15–30	50,000–150,000	80,000	1000–6000	...
Yellowtail	*Seriola dorsalis*	...	Liver	...	50,000[b]	...	17,000[b]	...

---

[a] Acknowledgment is made to Mr. William Hamm of the Fishery Technological Laboratory, U. S. Fish and Wildlife Service, Seattle, Washington, for compiling this table.

[b] These results represent a single sample or limited data.

mono-unsaturated oleyl alcohol), are present in relatively large quantities in ratfish-liver oil. Waxes and fatty alcohols occur principally in the head oils of certain marine mammals such as whales. The principal hydrocarbon in marine oils is squalene ($C_{30}H_{50}$) containing six unsaturated bonds. It is an important component of various fish oils, especially some species of shark- and dogfish-liver oils where it may represent one-third or more of the whole oil.

6. Vitamin Content

Fish-liver oils represent the best commercial source of vitamin A and some species, especially the tuna fishes, are also an excellent source of vitamin D. With many species, oil of high vitamin A and D content can also be obtained from portions of the viscera other than the liver. The body oils of fish are as a rule poor sources of vitamins.

In Table 85 are presented data on the vitamin A and D content of various fish oils and also an indication of the per cent oil present in the liver in each case. No values are included to show the relative size of the liver as compared with the size of the whole fish since insufficient data were available. In general, the livers of the true fishes (Teleostomi) range between 1 and 5% (usually between 1 and 2%) by weight of the total weight of the whole fish. The weight of the livers of the sharks and related species (Elosmobranch) usually represents between 5 and 15% of the entire weight of the whole fish.

The data in Table 85 have been compiled from many sources. Most of the information was taken from published and unpublished data of the Technological Fishery laboratories of the U. S. Fish and Wildlife Service (formerly Bureau of Fisheries) and from Bulletin No. 59 of the Fisheries Research Board of Canada.[8] Many other values reported in the literature have been considered and those values selected which were believed to be most accurate. In most cases the ranges reported were compiled by considering data from several sources. The results presented in this table cannot be considered to be final or entirely accurate since data from such widely varying sources as laboratory analyses and commerical operations had to be used for different species. It was attempted, as nearly as possible, to include in the vitamin range columns, the range of values which might be encountered in commercial operations due to season at which fish were caught or other similar factors. In some cases, an average vitamin content is also indicated. These values were included where a rather large range of vitamin content would be encountered and where fairly definite indications were available as to the average values to be expected.

Owing to the pre-eminent position of fish and especially fish-liver oils as a source of vitamins A and D, it is often overlooked that sea foods are also rich in certain other vitamins. Very few analyses have been made on such vitamins in fish products. The most extensive investigation of this kind is that carried out by the Research Laboratory of the Norwegian Canning Industry by Lunde[9] and coworkers.

---

[9] G. Lunde, *Angew. Chem.*, 52, 521 (1939).

Results obtained by them and in a few cases by other workers are presented in Table 86.

Fish flesh is a fair source of thiamine, being somewhat poorer in this vitamin than most meats. Shellfish and crustacea probably have a higher thiamine content than fish. Thus Fellers and Harris[10] report Atlantic crab to contain 230 γ thiamine per 100 g. crab meat, a value comparing very favorably with those of meats. Fish roe is a very excellent source of thiamine, ranking with the best food sources in this respect. Fish liver also contains relatively large quantities of this vitamin.

TABLE 86

WATER-SOLUBLE VITAMINS IN FISH AND FISHERY PRODUCTS[a]

Substance	Ascorbic acid (vitamin C), mg. per 100 g.	Thiamine (vitamin B_1), γ per 100 g.	Riboflavin (vitamin G), γ per 100 g.	Pyridoxine (vitamin B_6), rat units per100 g.	Niacin (nicotinic acid mg. per 100 g.)
Mackerel, flesh	..	120	...	...	...
Flounder, flesh	..	105	...	...	...
Cod, flesh	..	60	160	50	2
Herring, flesh	..	30	...	100	3
Haddock, flesh	..	...	...	100	0.9[b]
Salmon, red, canned	..	...	220[c]	...	...
Brisling sardine	..	...	600	100	...
Mackerel roe	40	600	...	...	...
Cod roe	30	1200	1000	...	1.5
Pollock roe	30	...	...	...	...
Herring roe	20	30	...	...	...
Haddock roe	10	...	...	...	...
Brisling sardine roe	10	...	...	...	...
Salmon roe	..	1200	1000	...	...
Cod liver	..	300	800	400	1.6
Cod milt	..	180	400	100	...
Herring milt	..	...	500	50	...

[a] G. Lunde, *Angew. Chem.*, 52, 521 (1939).
[b] W. J. Dann and P. Handler, *J. Nutrition*, 24, 153 (1942).
[c] H. E. Munsell, *Food Research*, 7, 85 (1942).

Of considerable interest is the relationship between consumption of certain whole fresh-water fishes by foxes or other fur-bearing animals to the thiamine content of the diet. Thus Green, Carlson, and Evans[11] report that foxes fed whole carp developed Chastek paralysis. This disease failed to develop, however, when the diet was supplemented with 10 mg. or more of thiamine per day. The disease did not develop following feeding upon carp flesh only, nor did it occur if whole carp were fed intermittently for several days a week and a diet containing adequate thiamine on other days. The hypothesis has been advanced that some substance present in the whole fish has a "sparing" action on thiamine in the feeding of foxes similar to that for the consumption of large quantities of alcohol by man.

Fish flesh is a good source of riboflavin, ranking about the same as meat, and fish roe and fish livers are extremely rich sources of this vitamin. Billings, Beely, Fisher, and Hedreen[12] have shown that commercial fish meal contains extremely high quantities of riboflavin. Thus samples of commercial pilchard, salmon, and herring meals assayed between 900 and 2200 γ per 100 g. of meal. Special meals prepared from the livers of salmon, tuna, pilchard, and herring contained (on a moisture- and fat-free basis) 5000 to 10,000 γ per 100 g. of meal.

---

[10] C. R. Fellers and S. G. Harris, *Ind. Eng. Chem.*, 32, 592 (1940).
[11] R. G. Green, W. E. Carlson, and C. A. Evans, *J. Nutrition*, 23, 165 (1942).

As shown in Table 86 fishery products are a very good source of vitamin B_6 and a fairly good source of niacin.

7. Miscellaneous Substances in Fish

In addition to the principal nutritive components of fish, protein, minerals, oil, and vitamins, there occur many other substances far too numerous to completely list here. Numerous nonprotein nitrogen compounds are present. In addition to free amino acids, various basic nitrogen compounds occur. Beatty[13] reports various Teleost salt-water fishes to contain an average of 0.31 to 0.67% trimethylamine oxide in the press muscle juice. In dogfish an average of 1.25% was found to be present. Fresh-water fish were shown to contain no trimethylamine oxide. The oxide decomposes during spoilage to give trimethylamine and small amounts of dimethylamine. Creatine phosphoric acid is reported by Zagami[14] to be present in the muscle of fish in quantities ranging from 0.1 to 0.6% and it is shown that there is a correlation between the content of this substance and the muscular activity of the fish, those migrating over the larger distances having higher content of the phosphagen.

Small quantities of formaldehyde have been reported in fish, especially in canned fish. Early investigators attributed its presence in canned fish to slow oxidation of trimethylamine which led to increasing concentrations over prolonged storage of the canned product. While such a reaction may take place, the presence of formaldehyde in fresh (uncanned) fish has been shown by Lunde and Mathieson.[15]

Pigments occur in the flesh and oil of some species of fish. Bailey[16] has reviewed the literature and contributed some experimental work on the pigments in salmon. The red pigment is identified as a form of astacin, also known to occur in the lobster. Beely and Chalmers[17] believe the principal yellow pigment present in pilchard oil is fucoxanthin. Other pigments believed to be present in small quantities in some fish include carotene and chlorophyll.

Glycogen is present in the flesh of fish, and, as in mammals, it functions as a source of stored energy, yielding lactic acid through a series of reactions. Oysters contain especially large quantities of glycogen (up to 2 or 3% in the meats); other species contain a few tenths per cent. The transformation to lactic acid, completely reversible in the living fish, becomes irreversible after death so that as a result of post-mortem changes, the lactic acid content rises from a few hundredths per cent in the living muscle to a value of 0.3 to 0.5% when full *rigor mortis* is attained.

---

[12] F. Billings, J. Beely, H. Fisher, and C. Hedreen, *J. Nutrition*, **22**, 425 (1941).

[13] S. A. Beatty, *J. Fisheries Research Board Can.*, **4**, 229 (1939).

[14] V. Zagami, *Atti accad. Lincei*, **10**, 599 (1929).

[15] G. Lunde and E. Mathieson, *Ind. Eng. Chem.*, **26**, 974 (1934).

[16] B. Bailey, *J. Biol. Board Can.*, **3**, 469 (1937).

[17] J. Beely and W. Chalmers, *Proc. World's Poultry Congress, 6th Congr.*, Berlin and Leipzig, **1936**, p. 228.

The *p*H of the flesh of most species of fish is between 6.6 and 6.8 immediately after death. As lactic acid accumulates the *p*H falls, but owing to the excellent buffering action of the flesh, the decrease is not great, and in the case of the muscle of fish, a drop below *p*H 5.8 is exceedingly rare. As spoilage takes place, an accumulation of basic end products such as trimethylamine and ammonia occurs so that the *p*H then rises, slowly at first and then quite rapidly, reaching a value of 7.5 to 8.0 in extreme spoilage. In the case of oysters no rise in *p*H occurs, since spoilage in this species takes place as a souring of the product due to accumulation of large quantities of lactic and other acids. In extreme spoilage the *p*H of oysters may fall to 4.8 or lower.

III. SPOILAGE OF FISH

Since fish are the most perishable of all flesh foods, the problem of spoilage and its prevention is of great practical importance. The reactions involved are exceedingly complicated and since no really comprehensive studies have been made on this subject, our knowlege in this field is still very limited. Unfortunately the largest portion of the investigations made have been directed toward finding a test for determination of the amount of spoilage which has taken place, rather than to investigate the exact nature of spoilage. This has resulted in much work having been done on certain reactions involved in spoilage which lend themselves readily to analytical methods of determination, although such reactions may be of relative insignificance to the actual spoilage processes.

In general, spoilage usually proceeds according to the following pattern. Shortly after death of the fish the glycogen in the muscle is converted into lactic acid and *rigor mortis* sets in. This phenomenon is accompanied by a decrease in *p*H, and a transformation of the flesh from the soft semiliquid state of the living tissue to a gelatinous condition. Both these conditions are unfavorable to bacterial decomposition of the tissue, and so long as rigor persists, such bacterial action is greatly retarded if not stopped entirely. During rigor certain autolytic changes may proceed causing a softening of the flesh and a general breakdown of the nitrogenous components. In fish which have not been eviscerated, such autolysis may be considerable and a general softening of the flesh adjacent to the visceral cavity occurs. In some instances especially when the fish has been feeding on certain organisms (such as "red feed") the softening of the flesh may be accompanied by hydrogen sulfide formation. In the case of dressed fish where the viscera is absent, this autolytic stage is of considerably less importance and may not be evident at the time, but it contributes to a later, more rapid bacterial decomposition.

After rigor has subsided bacterial spoilage proceeds at a continually accelerating pace until spoilage is complete. Sometimes in the later stages of spoilage or especially in cases where bacterial changes are retarded by preservative methods (*e. g.*, in salt fish or frozen fish), oils, if present in the flesh, become oxidized and rancidity develops.

The protein of fish is first broken down into intermediate amino acids by the action of enzymes and bacteria, although in ordinary types of fish spoilage this decomposition is generally a bacterial rather than an autolytic process. At this stage the fish is still in relatively fresh condition although subject to rapid alteration to undesirable end products such as hydrogen sulfide, indole, etc.

Certain nonprotein nitrogenous compounds are present in fish flesh some of which play an important part in spoilage, although present in relatively small amounts. One such compound is trimethylamine oxide, which on decomposition yields trimethylamine which possesses an odor characteristic of stale fish. Much work has been done by the Fisheries Research Board of Canada on the role of this compound in fish spoilage. They have shown that the increase in trimethylamine is a good measure of the degree of spoilage. According to the experience of the writer, while the trimethylamine content can be used as an index of spoilage, under certain unusual conditions samples may occasionally be encountered which contain large quantities of this substance (equal to that associated with complete spoilage) without giving any stale odor. Accordingly, the presence of this compound cannot be considered as the principal cause of spoilage but only one of many end products all of which contribute to the odors and flavors associated with spoilage. Other such compounds which have been used as an index of fish decomposition include ammonia, dimethylamine, hydrogen sulfide, and indole.

Fish oils, being highly unsaturated, are very susceptible to oxidations and rancidity. Such rancidity follows the usual induction curve; that is, a dormant period is followed by a very rapid increase in rancidity. As with protein spoilage, the oxidation of fish oils is an exceedingly complicated process making it impossible to depict the complete reaction involved and products formed. Hydrolysis of the oil to free fatty acids occurs but does not seem to be of much significance in the development of rancidity of fish oils.

IV. FISHING METHODS

Fishing gear can be broadly divided into three classes: that using lines; that using nets or seines; and that employed largely in the taking of shellfish and crustacea and consisting of miscellaneous methods. Each of the first two classes can be divided into two subclasses, the first in which the gear is more or less stationary or drifts with the tide, and the second in which the gear is hauled by power through the water.

1. Lines

The simplest type of line gear is the hand line, consisting of bait, hook, line, and sometimes a pole. This method was formerly one of the principal methods used by the extensive North Atlantic fisheries, but today it has been largely superseded in this area by other methods. Hand-line fishing is today most important in the fishery for red snapper in the Gulf of Mexico and for tuna in the Pacific. In the red snapper fishery bait consists of "alewives" or "skipjack" purchased fresh and salted on the fishing vessel. The tuna fleet employs live sardines carried in tanks aboard the vessels. Fishing takes place either from small dories or directly from the fishing vessel.

Fig. 41.—Fishing for tuna.[17a]

Trawl lines or line trawls consist of "ground lines" laid horizontally near the bottom of the sea and marked and held in place at each end and at intervals by buoys. Ganging lines, short lines attached at right angles to the ground line, carry the hooks. The gangings are fastened to the ground line at intervals of from 5 to 10 ft. Bait consists of such fish as mackerel or herring which usually has been frozen until placed aboard the fishing vessel. Trawl lines were formerly the principal gear used for taking such New England ground fish as cod, haddock, etc. They are still used to a considerable extent in this fishery. Use of line trawls is the chief method employed by the halibut fishery, and this gear is also used extensively on the Pacific coast for taking other "ground" fish such as the rockfishes, grey cod, and sablefish.

Troll lines (not to be confused with trawl lines) are used extensively on the Pacific coast for taking of king salmon, silver salmon, and albacore tuna. The lines pass from a power gurdy up over poles which slant outward and upward above the boat while the line is towed slowly through the water. In Alaska, the number of lines per boat is limited by law to four.

2. Nets and Seines

Pound nets or traps consist of (*1*) a leader or long net extending from the shore or from shallow water out to the main part of the trap in deeper water, (*2*) the heart, a circular or heart-shaped net leading into (*3*) the crib or pot, a rectangular net in which the fish accumulate. Fish swimming along the shore reach the leader and are deflected into the heart and then into the crib or trap proper from which they are unable to find their way out. As the trap becomes filled with the live fish, they are brailled out into scows or boats. This type of gear has many variations, but the principle of operation is the same. The trap may be anchored firmly to the bottom by means of stakes or piles, or it may be constructed as a floating trap. Simple varieties constructed from brush, branches of trees, etc., are known as weirs. Traps and weirs are used extensively on the Atlantic coast for herring, mackerel, whiting, sea trout, butterfish, alewives, and croaker. In Alaska, traps are one of the principal methods of taking salmon.

[17a] Figures 41–45 reproduced by courtesy of "*Pacific Fisherman.*"

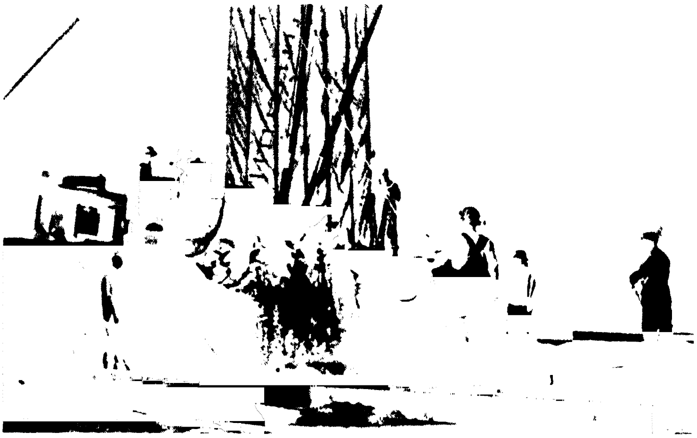


Fig. 42—Brailing a salmon trap. (Photo by Richard A. Ramme.)

Gill nets are rectangular nets set either just beneath the surface of the water if surface swimming fish are to be caught or near the bottom if ground fish are desired. The fish, swimming into the net, push their heads through the mesh, get their gills entangled in the webbing, and are unable to escape. The mesh size is determined by the size of the fish to be caught and may vary from an inch up to a foot. The gill nets may be anchored in place with stakes or allowed to drift with the tide. The gill net is the principal type of fishing gear used in the fisheries of the Great Lakes. This gear is also used to take important quantities of hake in Maine, pollock in Massachusetts, mullet and Spanish mackerel in Florida, and salmon in Oregon, Washington, and Alaska.

Purse seines and otter trawls represent the type of gear which is maneuvered into position to entrap or surround the fish. Purse nets consist of rectangular nets with buoys at the top and a purse line at the bottom such that the rectangular net is placed in the water in a circular shape, forming a hollow cylinder with the school of fish within, and then the purse line is drawn closing the bottom of net. One end of the net is commonly fastened to the main fishing vessel and the remainder placed in a smaller purse boat carried aboard the fishing vessel or towed behind. When a school of fish is sighted, the smaller boat maneuvers around the school, thus surrounding it with the net. The lampara net, used in California, operates on the same general principle as the purse seine. Haul seines are smaller seines operated in a similar manner but in shallow water, near shore or in rivers or lakes. The net is usually placed so that the fish are between it and the shore, and the ends then brought in to shore. Sometimes one end of the seine is fastened permanently by stakes to the shore.

Fig 43 —Setting a purse seine

Otter trawls consist of large baglike nets shaped like a dirigible, but with one end open. This baglike net is towed behind the fishing vessel and near the bottom and becomes filled with fish. The open end is prevented from collapsing by use of two large boards or "doors." These boards, rectangular in shape, about 10 ft. by 5 ft. or larger, are towed behind the boat with the open end of the net then fastened to the boards in such a way that the pressure of the water against the boards holds open the net. Other similar gear include the beam trawl and the paranzella net. The otter trawl is the principal gear used in taking bottom feeding fish on the Atlantic coast. It finds its largest use in the New England cod and haddock fishery.

The purse seine, trawl line, and otter trawl account for nearly three-fourths of the annual catch and more than one-half of the total value of the catch of fish landed in the United States.

V. HANDLING FRESH FISH

1. On the Vessel

Precautionary measures required in handling fish aboard fishing vessels depend largely upon two factors. Of most importance will be the length of time required to transport the fish to shore and the temperature of the fish during transit. Naturally where fishing grounds are hundreds or thousands of miles from the point where the fish are to be landed or where tropical climates prevail great care in providing adequate refrigeration is necessary. The second and less obvious factor includes the species of fish and method by which it is caught. Small fish always are more difficult to keep in good condition than large ones. Thus fish such as halibut or tuna will keep much better than herring or smelt if similar storage conditions are used. Furthermore, methods of catching which permit the least alteration in the fish during catching provide fish of the best keeping quality. Fish caught in traps or purse seines are of best quality immediately after catching since there has been a minimum opportunity for bruising or other damage. Methods of catching which allow the fish to struggle excessively provide a catch of decreased keeping quality. Fish caught in gill nets often struggle for many hours from the time they are caught in the nets until taken aboard the fishing vessel. Such fish during their struggle have prematurely gone into *rigor mortis* and under such conditions rigor does not persist for normal duration, and keeping quality is diminished. Fortunately gill-net fishing always takes place reasonably close to the point where the fish are landed, so that fish taken by this method are generally in excellent condition. Fish caught in otter

Fig. 44.—Unloading a salmon scow.

trawls have not only struggled considerably but also may be bruised, both factors tending to reduce keeping quality.

Upon being taken aboard the fishing vessel some species are dressed and others stowed whole. In general, autolytic changes are greatly retarded by dressing the fish while bacterial decomposition is not greatly affected, but the latter is apt to be increased somewhat for dressed fish. Fish which have been feeding just before they are caught are most likely to undergo autolytic decomposition, apparently containing enzymes associated with the digestive process which are of greater activity than those in fish, which have not been feeding. Accordingly, where possible, fish which have been feeding are eviscerated as soon as they are taken aboard the fishing vessel. A good example of this practice is found in the salmon fishery. Chum, pink, coho, and sockeye salmon are taken largely near the end of the spawning migration at a time when the fish have not eaten food for a long time and these fish are landed whole. King salmon are taken throughout the year by trollers and since they often have been feeding, they are eviscerated before being landed.

Some species of fish which have been feeding when caught are very difficult to eviscerate. Such fish include those which are caught in huge quantities and which are quite small, as for instance, mackerel. In such cases, evisceration would interfere with fishing operations since the fish are taken aboard the vessel much faster than dressing operations can take place, and such fish are usually landed whole even though dressing them when caught would add considerably to their storage life.

Where any considerable time will elapse between catching and landing of the fish, the catch is iced as it is caught. For this purpose ice mechanically crushed, usually by passing blocks of ice through a crusher consisting of a rotating drum containing short spikes, is used. The crushed ice thus obtained consists of pieces up to the size of an egg. Such ice is used for all but the smallest, most delicate species in which case a special, finely crushed ice is employed which is obtained by using a crusher with more closely spaced picks in the rotating drum.

Fish and ice are mixed in widely varying proportions, depending upon species and size of fish, distance they are to be transported, and climatic conditions. Fish which will be landed within a few hours are generally stowed in the hold without ice. Those which require several days or more are carefully iced in bins, boards usually being placed between layers of fish and ice to prevent the weight of fish from crushing those at the bottom of the hold.

Where considerable handling of the fish aboard the fishing vessel takes place in such operations as dressing, icing, and stowing in the hold, a sharp tined fork is sometimes used. This practice is to be discouraged since each time the fork penetrates the flesh, bacteria present in large quantities at the surface and in the slime are given a chance to penetrate within the flesh at the point where the tine enters the fish. Since several fisheries are conducted without the use of such forks at all it should be possible to dispense with them completely and thus increase the quality of the fish considerably. At any rate where forks are used great care should be taken to use them only on the heads of the fish where bacterial contamination is of less importance.

2. At the Fishing Port

Upon arrival at the fishing port the fish are unloaded either on elevators, often of the endless chain, bucket type which can be lowered to the level of the fishing vessel or scow, or by means of baskets operated by hoists from the fishing vessel hold to the wharf. Here the fish are usually first weighed and then carted to the fish house for dressing and packing for shipment.

3. Marketing

Fresh fish are generally marketed in one of the following ways: whole or round, in which the fish are not dressed in any way; gibbed, in which the gills and viscera are removed without slitting the belly cavity; eviscerated, in which the viscera are removed by slitting the belly cavity, but gills are usually left intact; dressed, in

TABLE 87

PACKAGED FRESH FISH PRODUCED IN UNITED STATES IN 1939[a]

Species	Quantity, lbs.	Value
Blue pike, fresh fillets	2,354,000	$ 505,580
Cod, fresh fillets	9,326,000	1,017,000
fresh sticks	289,400	27,284
Croaker, fresh fillets	173,000	17,000
fresh pan dressed	280,000	20,950
Cusk, fresh fillets	1,272,000	130,900
fresh sticks	543,000	51,400
Flounders, fresh fillets	8,036,000	1,064,000
fresh steaks	85,000	19,750
Groupers, fresh fillets	238,900	36,165
fresh steaks	314,800	50,711
Haddock, fresh fillets	19,078,000	2,105,000
fresh sticks	64,000	8,250
Hake, fresh fillets	1,673,000	161,600
fresh sticks	625,800	58,330
Halibut, fresh steaks	136,000	20,460
Ling cod, fresh fillets	186,000	16,260
Mackerel, fresh fillets	738,000	136,860
Pollock, fresh fillets	2,833,000	241,900
Rockfish, fresh fillets	565,000	88,060
Rosefish, fresh fillets	4,027,000	455,500
Sauger, fresh fillets	1,415,000	311,840
Sea bass:		
Atlantic, fresh fillets	150,000	22,100
fresh pan dressed	88,000	9,200
Pacific, fresh steaks, black	165,000	28,800
fresh fillets, white	115,000	24,300
Snapper, red, fresh fillets	100,000	27,840
fresh steaks	50,400	11,300
Squeteagues (sea trout), fresh fillets	288,000	39,800
fresh pan dressed	350,000	23,400
Totuava, fresh steaks	850,000	212,500
Whiting, fresh fillets	88,100	9,660
fresh pan dressed	10,755,000	345,200
Yellow perch, fresh fillets	529,000	117,800
Yellow pike, fresh fillets	553,000	130,240

[a] R. H. Fiedler, U. S Fish and Wildlife Service, *Statistical Digest* **1** (1942).

which head, fins, tail, and viscera are all removed; as split fish, where the fish is split down the backbone, head and viscera removed, the two halves held together by the belly wall being left intact; fillets, in which the flesh is cut into two boneless strips one from each side and either with or without the skin removed; butterfly fillets, where the two fillets are left adhering together at the backbone; sticks consisting of uniform width sections of fillets; and steaks consisting of cross-section slices of fish cut at right angles to the backbone and about one-half inch thick.

Fish are usually packed directly in the same kind of crushed ice as is used on the fishing vessel, although with some small, delicate species, shaved ice (known sometimes in the fisheries as "dry ice" but not to be confused with carbon dioxide dry ice) is employed. The amount of ice used will depend on climate and distance to be shipped. Fillets or steaks were formerly packed in tin containers but since the tin shortage has become acute wooden or, in some cases, waxed cardboard boxes containing 10- to 25-lb. fillets and sometimes lined with moistureproof paper are employed. These boxes are then packed in large wooden boxes of crushed ice. Whole fish are packed in large wooden boxes of crushed ice; however, no standard size of box is prevalent in the industry. Wooden barrels are also being used though not so much as formerly. Fish are shipped by rail (usually express) or by truck. In the former case the fish are placed either in refrigerated cars having ice bunkers at each end or in the regular express cars. In the latter case the boxes of fish are frequently covered with cubes of dry ice and the latter inclosed with insulated tarpaulins. This practice greatly reduces melting of ice and eliminates necessity for re-icing in transit.

Table 87 shows quantities and value of fresh packaged fishery products produced in the United States in 1939.

VI. HANDLING FROZEN FISH

A widespread prejudice against frozen fish has been carried over from former years when refrigeration facilities were as yet not fully developed and when all too often freezing and cold storage were considered as the last resort for fish in incipient decomposition. This prejudice still prevails in many quarters. Freezing represents the preservation method by which fish can be kept not only for extended periods of time, but also in a condition more closely resembling the fresh fish than by any other method.

Fish are frozen either whole or in packaged form. The latter method, a development of the past 20 years, has been given considerable acceleration by the introduction of quick-freezing methods. Packaged frozen fish have the advantage over whole fish of elimination of handling such waste portions as head, fins, tail, and, in some cases, backbone. Such a method also makes a much more convenient and attractive method of handling and retailing the product.

1. Alteration Caused by Freezing and Cold Storage

When a fish is frozen and then immediately allowed to thaw the thawed fish differs somewhat from its condition before freezing. It is usually softer, the texture is more porous, and sometimes considerable liquid or drip has formed which is not reabsorbed by the fish. These conditions are less acute, the more rapidly the fish has been frozen.

Two theories have been advanced to account for these phenomena. According to one theory, when fish is frozen extremely rapidly the water tends to freeze within the cell, causing the cell walls to expand but not break, whereas in slow freezing the water diffuses through the cell walls and freezes between them in long sharp layers. In the former case the cells, not being broken, more readily reabsorb the water and more closely resemble the fresh unfrozen fish.

According to the other theory the phenomenon is a colloidal one. With quick-frozen fish the water and tissue solids are frozen more or less *in situ* with the solids distributed fairly homogeneously throughout the ice. With slow freezing the water freezes out in a relatively pure state leaving the nitrogenous portion as a separate phase. This permits coalescing of the particles and when thawing commences reabsorption of the water takes place only with difficulty. In the case of the quick-frozen fish, however, the particles, being practically in their original state, readily reabsorb the moisture. The latter theory probably more completely explains the observed facts although doubtlessly the mechanism involved in the first theory also plays a minor part.

Chemical changes also occur but they are evident only after considerable storage time has elapsed. Storage at temperatures substantially below the freezing point of water completely eliminates bacterial action, but autolytic changes proceed, although at very slow rates. While some slight changes in the nitrogen fraction of fish, caused by enzyme action, occur slowly, these are so minor as to have no harmful effect on the nutritive value and little effect upon flavor, even after prolonged storage for a year or more.

Changes of a somewhat different nature occur during cold storage whereby the nature of the protein is altered and the texture of the fish becomes tough. This has been explained as due to alteration in *p*H during storage which brings the acidity closer to the isoelectric point thus causing decreased solubility of the protein, and by an alteration in the protein to a modified "meta" form which is tougher and less soluble than in the original state.

Oxidation is one change which continues at a perceptible rate even at low storage temperatures. This results in development of rancidity in oily fish, fading or other changes in the pigments of fish such as salmon having colored flesh, and development of off colors (usually yellow or brown) known as rust in other cases. The latter change is sometimes explained as a combination of the action of oxygen and ammonia on the oils, the ammonia coming either from a faulty ammonia compressor or refrigeration equipment or from the decomposition of fish which were somewhat stale when first placed in cold storage. Changes due to action of oxygen of the air are retarded by providing suitable protective means to keep air away from the fish. All types of changes occurring during cold storage are reduced to a minimum by using as low a storage temperature as feasible.

2. Freezing Methods

Fish were originally frozen by merely placing them in the cold storage rooms. Large fish require, under such conditions, several days to freeze completely. Such practice results in raising the temperature of the entire storage room and thus causes damage to fish previously frozen and stored there. To avoid these difficulties practically all fish-freezing establishments are provided with some sort of "sharp" freezer. This usually consists of a room or rooms equipped with shelves constructed from pipes containing the refrigerating medium, covered with sheets of galvanized iron. The fish to be frozen are placed directly upon these shelves. In some sharp freezer installations rapid air circulation is accomplished by means of a blower, and with such equipment the rate of freezing may approach or equal that attained in other types of "quick"-freezing equipment.

The first quick-freezing methods consisted of processes whereby whole fish were frozen by direct immersion in brine. Brine having a heat conduction approximately seven times as great as air is a more satisfactory freezing medium. Modifications were next introduced whereby the fish were placed in various metal pans or other protective devices to prevent absorption of brine. More recently many patented freezers have been used. For a review of such equipment see Lemon.[18]

One system which is the basis for several such patented quick freezers involves placing the packaged fish fillets or steaks between metal shelves which contain a circulating refrigerating medium having close contact with the shelves (sometimes with pressure on both the bottom and the top of the package of fish). With such systems packaged fish are frozen in from $^1/_2$ to 2 hrs.

3. Protection in Cold Storage

Before subjecting fish to cold storage some protective measure must be taken to prevent loss of moisture through evaporation and to keep out oxygen from the air. Whole fish are generally covered with a glaze of ice. The frozen fish are dipped in a tank of cold water whereupon a thin layer of ice freezes over the entire surface. It is of importance to have the temperature of the fish close to that of the glazing water in order to prevent cracks forming in the glaze due to rapid temperature changes. One alternate method of avoiding such cracks is to make use of various patented compounds which when dissolved in the glazing water form an ice glaze of superior quality which resists the tendency to crack.

Fish fillets are also sometimes frozen in blocks of 10 lbs. or more and then covered with an ice glaze. Such a glaze if kept intact furnishes a very excellent protection against access to air and any evaporation which takes place comes from the glaze rather than from the fish.

Whole, glazed fish are usually stacked like cordwood in the cold-storage rooms. At suitable intervals it is necessary to renew the glaze if prolonged storage is contemplated. This is most easily done by spraying water on the frozen fish.

---

[18] J. M. Lemon, U. S. Bur. Fisheries, *Investigational Rept.* **16** (1932).

Fish fillets are most commonly wrapped in some sort of moisture-vaporproof paper. The wrapper must be selected with care and must not only prevent evaporation of water through it but also must resist cracking at low temperatures, a defect common to many otherwise satisfactory wrappers.

Fish fillets or steaks are sometimes wrapped individually, packaged in cartons of about 5 lbs., and then frozen, while in some cases they are placed in 1-lb. cartons, frozen, and then wrapped. In other cases they are wrapped in moisture-vaporproof individual wrappers, frozen, and then packed in cartons.

VII. CANNED FISH[19]

Table 88 lists the principal fishery products canned in the United States in 1938. Since the packs of salmon and tuna represent about 70% of the value of all species canned in the United States and Alaska discussion of canning methods will be limited to these two species.

TABLE 88

PRINCIPAL CANNED FISHERY PRODUCTS PRODUCED IN THE UNITED STATES AND ALASKA IN 1938

Product	No. of canneries	Value of product	Quantity of product	
			Lbs.	Standard cases
Salmon:				
Alaska	98	$36,600,000	326,700,000	6,800,000
United States	27	5,700,000	22,700,000	470,000
Tuna	20	15,200,000	66,100,000	2,750,000
Sardines:				
California	31	7,100,000	109,000,000	2,250,000
Maine	25	2,370,000	16,800,000	670,000
Shrimp	50	4,870,000	18,100,000	1,075,000
Clam products	60	3,190,000	19,300,000	770,000
Mackerel	24	3,000,000	46,300,000	965,000
Cat and dog food	9	890,000	19,800,000	413,000
All other	..	4,480,000	23,200,000	837,000
Total	382	$83,400,000	668,000,000	17,000,000

1. Salmon

Five separate species of Pacific coast salmon are canned and these, with descriptive information, are listed in Table 89.

American salmon canning is carried out largely in five areas. The Columbia River industry centered at Astoria, Ore., puts up a major portion of the chinook pack, and a small quantity of other species. The second area on Puget Sound between Bellingham and Seattle puts up relatively small packs of each of the five species. Pink salmon is packed in this area in odd years (*i. e.*, 1935, 1937, 1939, 1941, etc.), the run of fish in even years being practically nil.

Southeastern Alaska from the southern Alaska border to the vicinity of Juneau accounts for the largest portion of the pink salmon pack, with fairly sizable packs of the other species also. Central Alaska puts up the second largest pack of both red and pink salmon as well as considerable medium reds. This area has principal packing centers around Kodiak Island, the Shumagin Islands, and Cook Inlet. The Western Alaska area, chiefly in Bristol Bay, accounts for the major portion of the red salmon pack.

---

[19] Much material for this section was obtained from an unpublished manuscript on fish canning by Norman D. Jarvis of the U. S. Fish and Wildlife Service.

TABLE 89

DESCRIPTION OF FIVE SPECIES OF SALMON BEFORE CANNING

Scientific name	Common name in can	Other common names	Names common to fishermen	Distinguishing marks of whole fish
Oncorhynchus nerka	Red salmon	Sockeye, blueback	Quinault redfish	No spots
Oncorhynchus tschawytscha	Chinook salmon	King salmon	Spring, tyee, quinnat	Back, dorsal fin, and tail covered with round black spots
Oncorhynchus kisutch	Medium red salmon	Coho, silver salmon	......	Faint spots on back, dorsal fin, and upper part of caudal fin
Oncorhynchus gorbuscha	Pink salmon	. . .	Humpback	Large, oblong, black spots all over tail and on back
Oncorhynchus keta	Chum salmon	Keta salmon	Calico, dog salmon	No spots

Scientific name	Average weight of fish, lbs.	Length, in.	Shape of body	Size of scales	Color of raw fish
Oncorhynchus nerka	$6^1/_2$	17–32	Symmetrical	Medium	Deep red
Oncorhynchus tschawytscha	22	24–60	Robust	Large	Red to white
Oncorhynchus kisutch	9	20–36	Elongated and flattened	Medium	Light red
Oncorhynchus gorbuscha	$4^1/_2$	15–27	Slender	Small	Pink
Oncorhynchus keta	9	25–37	Robust	Large	Light pink

Salmon are transported from the fishing grounds to the canneries by scows or boats known as tenders, although the most recent innovation has been the use of power scows for this purpose.

Fishing grounds are located so close to the canneries that fish are seldom more than 12 hrs. out of the water when they reach the cannery. It is, therefore, unnecessary to ice them. The fish are unloaded on elevators of the endless chain, bucket type which lift them into bins in the cannery. A number of bins are provided and the fish are graded according to species, sometimes also according to locality of capture and type of gear. On the Columbia River, chinook salmon are also sometimes graded according to color of flesh, freshness, and oil content.

Fish are cleaned by means of a machine known as the "iron chink" so called because its introduction replaced Chinese labor which formerly cleaned the fish by hand. The fish pass from the bin along a conveyor to the "iron chink" where the head is first removed by a revolving knife, after which the body is caught between two rotating drums which hold it with the back down and tail forward while rapidly rotating saws and knives split the belly and remove the belly cavity. Waste material such as heads and viscera drop through a hole in the floor onto a conveyor or sometimes directly on a scow floating beneath the cannery.

From the cleaning operation, a conveyor carries the fish through a tank of water where they are washed and small pieces of fins or viscera or other remaining refuse are removed by hand. Loss during cleaning varies between 25 and 35%. One "iron chink" can dress and clean 3600 fish an hour, and the process is completely automatic from the time the whole fish enter the machine. This device is now used for cleaning all salmon to be canned except Columbia River chinook which are cleaned by hand.

The cleaned salmon are brought to the cutting machine where each fish is placed manually upon ledges or buckets which are slotted crosswise to receive rotary gang knives. These knives cut the fish into sections of the proper size to fill a can, as the buckets containing the whole fish move past the knives on an endless chain. Tail portions are usually saved and cut by hand to furnish small pieces to complete filling of underweight cans.

Cans used in Alaskan salmon canneries are generally shipped in a collapsed form without bottoms, thus saving storage and shipping space, an important consideration in Alaska where shipping costs of preformed empty cans would be as great as the final product owing to water freight being handled on a volume basis. The collapsed cans can be shipped 360 to a case which holds only 48 completed cans.

The collapsed cans are run through a re-former usually on the floor just above the canning line. After being expanded to cylindrical form a second machine puts a flange on each end after which a third one attaches the bottoms. The cans then roll down a chute to the filling machine where the appropriate weight of salt ($^1/_4$ to $^3/_8$ oz. per lb. of salmon) is added. Pieces of salmon from the cutting machine enter the filling machine and pass into a measuring box of the same size and shape as the can. Rotating knives cut off any surplus fish and then a plunger pushes the salmon into the can beneath, an accurately timed mechanism known as the vent rod simultaneously operating to permit the air to escape as the fish enters the can. In the latest salmon-canning equipment the cutting and filling machines operate as a single unit. Modern filling machines operate at speeds of 200 cans per minute, sometimes slightly faster.

Many canneries make use of an automatic weighing machine which rejects cans coming from the filling machine which are under weight. Inspectors add small pieces of fish to bring the weight to the required amount, trim off ragged edges, and repack improperly filled cans.

Cans pass into a "clincher" which attaches lids loosely and then the cans enter the vacuum-closing machine which contains a chamber in which part of the air is removed and the lid is sealed on tightly. After passing through a washer where jets of water shoot against the cans, they are arranged on trays, 168 lb. cans No. 1 tall to a tray, and from 6 to 7 trays or "coolers" are piled on a small truck or car. These are rolled on rails into the horizontal retort which holds from 3 to 10 cars and up to 10,000 cans. The retort is then closed and the salmon processed ordinarily at 10 lbs. steam pressure (240° F.) for about 90 min. for 1-lb. cans, a longer or shorter period for larger or smaller ones.

Cans from the retort pass first through a lye bath to remove fish oil or flesh and are then washed with water. The cans on the "coolers" are next allowed to stand with space between to permit rapid cooling and thus prevent overcooking. Cans are packed 48 1-lb. cans to a fiberboard carton or a proportionately larger or smaller number of cans of other sizes to make 48 lbs. per standard case although in practice the smaller-sized cans are often packed in much smaller cartons. Labeling is usually done in the warehouse and not at the cannery, and most of the Alaska pack are labeled in Seattle.

2. Tuna

Four species of tuna—yellowfin, bluefin, skipjack, and albacore, the last named the only one which can be labeled white meat tuna—are packed, and in addition bonito and yellowtail, two similar species, are put up tuna style. The tuna canning industry centers at San Diego and San Pedro, although much of the albacore is packed farther north at Monterey and Astoria, Ore. Unlike the salmon canning industry where fishing grounds are close to the canneries, the tuna industry sends its fleet out thousands of miles, fishing as far south as the equator. Since fishing vessels must be gone for many weeks adequate preservation of the fish becomes a considerable problem. The larger vessels freeze their fish which are first precooled in sea water cooled by artificial refrigeration to 28° F., and then in brine at 10° F. Smaller craft which fish closer to the canneries pack their fish in ice and where ice is carried, artificial refrigeration is sometimes used to retard the melting of the ice.

After arrival at the cannery, frozen fish must be thawed. Some canneries allow the fish to stand in air, rinsing them occasionally with water from a hose, while others immerse the fish in tanks of running water. Thawing requires 2 to 8 hrs. in running water, depending on the size of the fish, and 6 to 48 hrs. in air.

Tuna are butchered by hand, the viscera being removed but head and fins left intact. The dressed and washed fish are placed belly down in wire baskets on carts containing racks made of angle iron. Each basket holds one large or up to six small fish. The racks are then wheeled into steam chests and given a precook of from $1^1/_2$ to 8 hrs. depending on the size of the fish, using a temperature of 210 to 220° F. During the precooking period considerable oil and water are removed and an average loss in weight of 25 to 30% takes place. The precooking process is used primarily to remove excess moisture and oil which otherwise would give the pack a soggy texture with too much strongly flavored tuna oil present. The precooking also makes it much easier to remove the flesh from the bone.

The precooked fish are cooled for a period of about 12 hrs. The fish are next cleaned by hand, removing head, tails, and fins, and then scraping away the skin and separating the body in half. The backbone and rib bone are then removed and the halves split longitudinally permitting removal of the dark meat running along the lateral line in a V-shaped layer.

The cleaned white meat, carefully inspected to be certain all dark meat and refuse have been thoroughly removed, is next cut across the grain by means of an automatic slicer usually of the guillotine type. For half-pound cans slices one inch or slightly thicker are cut. The meat is graded as fancy, consisting of all large pieces, standard, about 75 to 85% large pieces, with the remainder made up of smaller fragments, and flakes, made up of all finely divided meat. Ordinarily one cannery puts up either the standard pack or both fancy and flakes. There is no difference in the quality of the meat among the different grades except for the size of the pieces.

Cans are filled by hand and well over three-quarters of the pack is put up in half-pound tuna (307 × 113) cans. Salt is next added, about $^1/_6$ oz. per half-pound can, and then winter-pressed cottonseed oil at a temperature of 180 to 200° F. usually about $1^1/_2$ oz. to a one-half pound can. Most canneries use an exhaust box for removal of air from the cans although about 30% are now equipped with vacuum closers. In the latter case the oil is not heated before being added to the can. The filled, sealed cans are given an alkaline wash and then processed in horizontal retorts. Processing usually takes place at 240° F. using 75 min. for the half-pound tuna cans. Cooling is carried out within the retort by shutting off the steam and admitting air under pressure followed by a spray of water. After 30 min. of cooling in the retort the cans are removed and held an additional 24 hrs. before labeling.

3. Quality and Inspection

Final quality of canned fish depends upon (*1*) the condition of the raw fish as received at the cannery, (*2*) methods used during canning, and (*3*) subsequent changes which may take place during storage after canning.

Several factors influence quality of fish prior to canning. The fish should be fresh when it reaches the cannery. With some species such as salmon which need to be transported only a short distance from fishing grounds to cannery it is a simple matter to provide fresh fish, but with other species such as tuna considerable care must be taken properly to refrigerate the fish and even under optimum conditions some spoilage may take place in which case adequate inspection and rejection of stale fish are necessary.

Even though fresh fish are used, the quality is not always satisfactory. In the case of salmon if the fish are taken late in the season when they are sexually mature the fish may be soft, water-marked, and often have very low oil content. Other species of fish which are feeding when caught may give difficulty when certain types of food such as "red feed" have been consumed so that even though the fish are fresh, considerable softening may occur before canning begins. Certain plankton upon which salmon feed sometimes give a musty or grassy flavor to the fish. Clams and other shellfish occasionally feed upon substances which render them poisonous and in such cases cannot be canned during certain seasons.

Errors in processing time or temperature are exceedingly rare in commercial fish canning but other less obvious details may decrease the quality of the pack. Some of these factors are proper cleaning of the fish, cans properly filled, correct headspace, correct amount of salt added, and adequate vacuum. Occasionally even though fish are properly processed they are cooled too slowly which in effect increases the processing time. This sometimes occurs by stacking the cans close together before they are cool and results in "stacker burn."

A number of products, especially shellfish, give considerable difficulty in becoming dark or discolor the can upon storage. Such products may contain sulfur in a form which readily releases hydrogen sulfide and this reacts with iron in the can to give black iron sulfide. A number of remedies are available for diminishing or eliminating this difficulty. The use of an enamel inside the can is of considerable help. Sometimes a special enamel containing zinc is employed. The zinc tends to form white zinc sulfide before the iron is attacked. With some products the use of parchment paper liners in the can is necessary to prevent darkening completely. Shellfish are often dipped in acetic, citric, or tartaric acid, and sometimes in sodium acid phosphate solution prior to packing. This lowers the *p*H and reduces the possibility of iron sulfide formation For crab meat the final *p*H of the meat should be between 6.5 and 7.5, higher *p*H values tending to accelerate the iron sulfide formation. Another difficulty encountered with crab meat is the tendency to develop a blue discoloration. This is caused by copper, which is present in the crab's blood in the hemocyanin (analogous to iron in hemoglobin), becoming oxidized and reacting with ammonia in the flesh to form the bright blue copper ammonia complex. One patented process to prevent this discoloration[20] involves brining the meat in a solution containing small amounts of aluminum or zinc salts.

Canned fish are inspected both by voluntary inspection service of the industry and by government agencies The salmon pack undergoes inspection at Seattle by the National Canners Association laboratories under a voluntary agreement among the packers. In addition to detecting and rejecting any lots which might not pass subsequent U. S. Food and Drug Administration inspection, this service also keeps records on other improvements which the canner might make to increase the quality of his pack, and this information is passed on to the individual packers.

In California, the State licenses the canneries and provides specifications for processing times and temperatures for each species of fish canned. Automatic temperature control and recording are required. State officials inspect the raw fish entering the cannery and can reject any which are not of sufficiently high quality.

Federal Food and Drug officials inspect canned fish entering into interstate commerce. Owing to the efficient State and voluntary inspection services only a very small portion of the pack is ever seized by the federal authorities.

---

[20] C. R. Fellers and S G. Harris, *Ind. Eng. Chem.*, **32**, 592 (1940).

VIII. SMOKED FISH

In past years when such modern methods of preservation as canning, icing, and freezing were unknown, fish were smoked primarily as a means of preservation. The tendency was to produce a heavily smoked product which would keep for weeks regardless of the undesirable bitter smoke flavor. Today with the many modern preservation methods available fish can be given a light smoke which enhances their flavor and then can be preserved by freezing, canning, etc.

Table 90 shows quantities and value of fish smoked in the United States in 1939 for species of which more than 100,000 lbs. of smoked product were produced. Species for which more than a million pounds were smoked include salmon, chub (including cisco and tullibee), sea herring, whitefish, and haddock.

TABLE 90
SMOKED FISH PRODUCED IN THE UNITED STATES IN 1939[a]

Species	Quantity, lbs.	Value, dollars
Alewives	218,000	11,600
Buffalo fish	367,000	96,900
Butterfish	566,000	148,000
Carp	252,000	83,000
Chub, cisco, and tullibee	6,280,000	1,940,000
Cod, smoked fillets	681,000	82,300
Cusk, smoked fillets	630,000	50,700
Eel	170,000	41,900
Haddock, smoked fillets	49,100	11,000
finnan haddie	990,000	100,000
Herring, lake	865,000	96,500
Herring, sea, hard bloaters	475,000	54,800
soft bloaters	100,000	5,500
unclassified bloaters	115,000	10,100
boneless	1,900,000	195,000
lengthwise	66,300	3,900
medium scaled	192,000	11,500
kippered	532,000	63,000
unclassified, whole	100,000	11,800
Lake trout	743,000	222,000
Mackerel	278,000	45,000
Paddlefish or spoonbill cat	153,000	77,400
Sablefish	460,000	130,000
Salmon, smoked	9,770,000	3,050,000
kippered	2,780,000	680,000
Shad	114,000	24,000
Sturgeon	972,000	655,000
Whitefish	2,760,000	866,000
Whiting	101,000	13,200

[a] R. H. Fiedler, U. S. Fish and Wildlife Service, *Statistical Digest* 1 (1942).

Smokehouses in general use in the United States have not shown much change in recent years. The larger installations generally consist of a tall compartment some times several stories high with removable racks, access to which is available through doors located on different levels or floors of the building in which the smokehouse is located. The fire is built at the bottom and the smoke rises through the fish hanging on the racks.

Recently improved, modern smokehouses have been made available for smoking meats. Such houses have provision for accurately regulating humidity, temperature, smoke density, etc., but such equipment has not been widely adapted to fish although it is admirably suited for fishery products. One such smokehouse has been developed by the Fishery Research Board of Canada, and has been successfully used commercially by a number of Canadian fish smokers.

Fish for smoking are generally split down the backbone. A few species are smoked whole, while in other cases fillets or other cuts are prepared. The dressed fish are first brined in a salt solution, the time of brining depending upon the product being prepared and varying from $^1/_2$ hr. to 72 hrs. or more. The purpose of the brine treatment is to add the desired salt taste to the fish and in some cases to improve its keeping quality by reducing the moisture content.

The fish are next dried, usually by hanging them in the smokehouse. With the newer, improved equipment a current of air of preadjusted humidity is drawn over the fish, both hastening the drying process and providing a uniform product independent of weather conditions.

Smoke is generally produced from a smoldering fire of mixed sawdust and chips or shavings. The variety of wood used varies with the locality. Thus on the Pacific coast alder is much used while in the East and Middle West the harder woods such as oak or hickory are favored.

The fish are usually first smoked at a relatively low temperature (80 to 150° F.). The time of smoking varies from 3 to 5 hrs. for such lightly smoked products as finnan haddie, to 2 weeks or more for the harder smoked fish. With many products, the fires are built up at the end of the smoking process to produce a higher temperature (usually about 260° F.), thus cooking the fish.

The improved keeping quality of smoked over fresh fish depends upon a number of factors only one of which is the presence of the smoke components. Some smoked fish such as bloater herring are heavily brined before smoking so that if the smoking process were to be eliminated entirely, the fish would still be well preserved. With many products the fish are not only brined but also given a thorough preliminary drying which adds further to the keeping quality by removal of moisture. This preliminary salting and drying probably contributes more to the preservation of the fish than does the actual smoking process.

Substances absorbed by the fish during smoking which add to its keeping quality include organic acids such as acetic and formic, formaldehyde, cresols, phenols, and similar compounds.

IX. SALTED AND DRIED FISH

1. Salted Fish

This means of preservation, although of secondary importance in the United States, is still the principal method used in many other countries. Salt acts not so much as a preservative itself but rather as a means of removing water from the flesh. By osmosis, water exudes through the cell walls of the fish flesh and salt solution passes in until equilibrium is attained and the concentration of salt within the cell is equal to that of the external brine. The water content is thus reduced to a point where bacterial and enzymatic actions are arrested or reduced to a very small rate. Salting of fish does not retard oxidation of fish oils so that such changes proceed slowly, and oily, salted fish may soon become rancid, unless protected by other means.

Fig 45 — Preparation of mild cured salmon This form of lightly salted salmon is an intermediate product which is used for preparing smoked salmon King salmon are mild cured on the Pacific coast and in Alaska, and are kept under refrigeration until they reach New York City where they are smoked

Most fish are salted in the United States by the "dry salt" process. The dressed and usually split fish are placed in a barrel or other container with salt placed between successive layers of fish. Upon standing, water is withdrawn from the fish to make a brine. Complete penetration of the salt is not obtained for about three weeks, and since spoilage may occur more readily in the partially cured fish than in the final salted product, it is necessary to take certain precautions to minimize spoilage and obtain maximum rate of penetration of the salt. Fresh fish must be used, and all blood should be removed in the cleaning operation. With some products the viscera are not removed, but such methods cannot be carried out successfully in a warm climate, since evisceration greatly retards spoilage. In warm climates it is also essential to use a pure grade of salt. Impurities such as calcium and magnesium salts retard the penetration of the sodium chloride. They also have the disadvantage of being retained when the fish is freshened and contribute a bitter flavor. Foreign impurities in the salt also sometimes accelerate discoloration of salt fish. Certain bacteria sometimes found around fish-curing plants tend to develop a red color on the fish and such discoloration is accelerated where impure salt, especially that having an alkaline reaction, is used. Use of pure salt and disinfection of wharfs and equipment reduce the chance of such discoloration.

TABLE 91

SALTED FISH PRODUCED IN THE UNITED STATES IN 1939[a]

Species	Quantity, lbs.	Value, dollars
Alewives, round	1,940,000	34,500
corned	5,753,000	126,000
pickled[b]	4,920,000	137,000
spiced	3,029,000	300,000
tight-packed cut	741,000	32,100
Blue runner, salted	112,300	4,750
Cod, green[b]	4,547,000	195,000
dry	1,700,000	122,000
boneless	4,528,000	737,000
Eels	155,000	11,680
Haddock, dry	51,800	2,580
boneless	101,000	15,300
Hake, green[b]	1,523,000	47,000
dry	651,000	27,150
Herring, lake	7,530,000	295,000
Herring, sea, pickled, Scotch cure	2,858,000	185,000
brine salted	114,000	5,000
dry salted	63,200	5,100
pickled in vinegar	984,000	55,500
Norwegian cure	32,500	2,800
roused	508,000	14,800
spiced	607,000	61,800
Mackerel, fillets	468,000	33,800
split, dry	739,000	48,300
Mullet	2,160,000	131,000
Pollock, green[b]	62,000	1,630
dry	542,000	27,300
Sablefish	1,171,000	72,400
Salmon, dry	8,720	726
mild cured	9,235,000	1,738,000
pickled	322,000	34,100
Spot	483,000	27,600

[a] R. H. Fiedler, U. S. Fish and Wildlife Service, *Statistical Digest* 1 (1942).
[b] Intermediate product.

Salt cod is produced by a combined process of salting and drying. The dressed, split fish with most of the backbone cut away are placed in large butts 3 ft. in diameter and 4 ft. high and mixed with about 7 bushels of salt. The butts are heaped high with fish, and after a day or so the fish sink down beneath the level of brine which has formed, and another bushel of salt is then added. Surplus brine is next removed by piling the fish on frames and weighting them down. They are then dried by placing them on frames in the open air. The action of the sun not only further dries the fish but also bleaches the color considerably. The fish require from a few hours up to a week for drying, depending upon weather conditions. In very hot weather a canvas is stretched above the fish to prevent undue action of the direct rays of the sun which may cause yellowing of the flesh.

Finally the skin is removed and the flesh picked by hand from the bone. Several grades are packed, and in some grades some of the smaller bones remain in the product. Before packaging a small amount of salt containing a few tenths per cent boric acid commonly is added to aid in prevention of reddening.

Table 91 shows amounts and value of salted fish produced in the United States

2. Dried Fish

Sun-dried fish, although common in some tropical countries, is not produced extensively in the United States. Dried shrimp is perhaps the most common fish dried in this way, and it is largely exported to the Orient.

There seems to be no doubt that by the use of modern drying equipment such as is employed in drying fruits and vegetables, an improved dried fish product could be obtained. By using controlled temperature and humidity a rapid dried fish product would be obtained with no chance for spoilage. By eliminating the use of salt the objection of many persons to the strong salt flavor of salted fish would be overcome. In the past no such product has been prepared commercially in the United States, possibly due to the exceedingly perishable nature of fish which precluded use of established methods for other products.

Recently as a wartime measure, interest in dehydration of fish has been revived and much research is being carried on at the present time by several government agencies. Present indications are that nonoily fish such as cod can be dehydrated in much the same way as meat. The fish are cooked, separated from bone, ground, and then dried in a stream of air such as in a tunnel drier.

X. FISH BY-PRODUCTS

Various by-products are produced from waste from the fisheries. These include fish oils, used both industrially and for feeding purposes, fish meal, used as animal feed and sometimes as fertilizer, glue, leather from fish skins, buttons from shells, and many other products. A brief discussion of those products which are used for food, especially for human consumption, follows.

1. Fish-Liver Oils

Cod-liver oil has been prepared for medicinal use for many years, long before the discovery of its vitamin A and D content. More recently it has been found that many other fish-liver oils exceed cod-liver oil many fold in vitamin content.

Methods of preparing liver oils differ according to the nature of the livers. Some species of fish such as cod, shark, and dogfish have livers which contain 50 to 75% oil. In such case rendering is a relatively simple process. It is only necessary to steam the livers to release the oil which is then separated from the water and nitrogenous material in separating tanks or, preferably, by means of a centrifuge. Many livers, especially those having very high vitamin potency, are of extremely low oil content and simple steaming does not liberate any appreciable quantity of oil. In such cases various patented processes are often employed. Perhaps the most common type of such process involves digestion of the liver with alkali which peptizes the liver cells and liberates the oil which is then centrifuged. Another process involves digestion of the livers with pepsin in acid solution followed by centrifuging. In some of these processes it is customary to add an oil of low vitamin potency to the digested liver to aid in removal of the vitamin by the solvent action of the added oil. Formerly solvent extraction was much employed but this method has been largely displaced by digestion methods.

The lower potency liver oils are much used as a vitamin supplement for animal feeds while the higher potency oils find favor for medicinal use or are employed in preparing vitamin concentrates. Such concentrates are usually prepared by removal of oil by saponification or by distillation of the vitamin using the molecular still. Cod-liver oil, although actually a low potency oil, is, through precedence, still much used for medicinal purposes.

2. Fish Body Oils

Fish body oils are prepared in conjunction with the manufacture of fish meal. Of special interest from the standpoint of human food is the recent practice of preparing salmon oil from the waste trimmings accumulated in salmon canneries and then adding the oil so obtained to the canned salmon. This practice has now become common on the Columbia River for canned chinook salmon and it is practiced to a small extent in British Columbia and in Alaska.

Trimmings from the edible portion of the salmon and sometimes the salmon eggs are cooked with steam, with or without the addition of alkali, and the oil is separated by centrifuging. The clear, pure salmon oil so obtained is then used to enrich the canned salmon, adding both to the appearance and to the nutritive value in the form of vitamin D and the calorific value of the oil. The formerly wasted salmon trimmings are thus utilized to increase the value of the salmon pack. Up to the present time this practice is restricted largely to the relatively expensive chinook salmon pack, since with the less expensive species the cost of adding oil is at present prohibitive.

3. Fish Meal and Oil

Fish meal is an important animal feed used for poultry, cattle, and sometimes for fur-bearing animals, for its all-around nutritive value of protein, minerals, and vitamins. The largest manufacturing industries utilize the menhaden on the east coast and the pilchard on the west coast of the United States. These fish are very poorly regarded for human food and the largest part of the catch is used for reduction purposes. In addition to the manufacture of fish meal and oil from these fish, trimmings from other species are also used. Thus in Boston trimmings from haddock, cod, rosefish, and other species which are extensively used for preparing fillets are collected and rendered into fish meal.

The customary operations in the preparation of fish meal include grinding the fish followed by cooking with steam. Oily species are next pressed and the oil and water subjected to further treatment in separating tanks usually followed by centrifuging to obtain the oil. The press cake is then dried usually in flame driers consisting of long rotary driers in which the fish are fed into one end in direct contact with flame produced by burning oil or other fuel. With some species a better quality meal is obtained by use of steam-jacketed vacuum driers; some of the more recent installations use this method. The dried meal is finally ground and sacked. The oil obtained is generally used for industrial purposes although in some cases (notably pilchard oil) it has been used as a source of vitamins A and D for poultry feeding.

XI. SHELLFISH AND CRUSTACEA

The average annual shellfish production of the United States is shown in Table 92. Shrimp leads in volume taken and oysters are first in value.

TABLE 92

AVERAGE ANNUAL SHELLFISH PRODUCTION IN UNITED STATES FROM 1935 TO 1939

Species	Annual quantity, millions of pounds	Annual value, millions of dollars
Oysters	87.2	7.8
Shrimp	136.4	4.3
Crab	94.4	2.7
Clams	69.5	2.9
Lobster	13.3	2.6
Scallops	8.8	1.2

1. Oysters

Although the United States leads in oyster production, extensive fisheries for this shellfish also exist in other parts of the world, notably France, England, China, Japan, Holland, and Italy. In this country the common eastern oyster (*Ostrea virginica*) is cultivated most extensively on Chesapeake Bay, and in the Gulf of Mexico, especially in Louisiana, although fairly extensive oyster beds are found all along the Atlantic coast south of Cape Cod. On the Pacific coast the native "Olympic" oyster (*Ostrea lurida*) is taken in small quantities on Puget Sound. Of more importance in this region, however, is the Pacific or Japanese oyster (*Ostrea gigas*) which has been introduced from Japan. The oysters taken on the Pacific, however, make up only about 10% of the total amount taken in this country.

Oyster culture has been developed to such an extent that in the care given in development of the oysters the industry resembles agriculture more nearly than fisheries. The selection of the site for the oyster beds must take into account many factors. With eastern oysters the water must be brackish with a salt content of specific gravity between 1.010 and 1.022. The bottom must not be too soft, since otherwise the oysters would sink and become covered, nor should it be so rocky and irregular as to interfere with dredging operations. It is desirable to have only small amounts of suspended matter in the water since large amounts of silt might smother the smaller oysters. The presence of large amounts of starfish, oyster drills, or other oyster enemies is also to be avoided where possible. The grounds should be located far enough from industrial plants which discharge waste material into the water to prevent contamination of the oyster beds.

During the development of the oyster larva, when a size of about 0.3 mm. is reached, the small oyster, "spat," or "set" as they are known at this stage, attempts to attach itself to a large oyster shell, rock, or other firm object. In modern oyster culture it is customary to aid this process by covering the grounds with special spat collectors. In this country oyster shells are used almost exclusively, usually several hundred bushels to the acre. In other countries various other objects are employed such as cement tiles, brush, and stones. As the oyster develops it reaches the seed oyster stage at which time it is commonly transferred to another area known as the growing grounds where conditions are most favorable for growth. After two or more years the mature oyster is generally moved once more to another area more suitable for fattening or development of a fine flavor, and it is then ready for market.

Oysters are removed from beds usually by one of two methods. The simplest means is the use of long-handled tongs from 12 to 25 ft. in length operated from small flat-bottomed boats. Dredges are used for larger scale operations. These consist of large rakes in front of a rope bag. The rake or "dredge bar" may be 24 to 72 in. in length, thus giving the bag a width of the same dimensions. As the rake is dragged behind the boat the oysters are loosened and fall into the bag.

Most oysters in the United States are shucked and sold fresh. They are graded according to size and on the Atlantic coast the larger sizes bring the highest prices while on the Pacific coast the smaller oysters are considered more choice.

2. Clams

Most clams are taken along the shores of the New England and Middle Atlantic States and especially in Massachusetts and Maine, although lesser quantities are produced all along both the Atlantic and Pacific coasts. Clams are not cultivated as are oysters but are taken from public beaches. They are usually dug with simple hand rakes, hoes, or similar tools. Since the center of the clam industry is in relatively heavily populated areas, contamination from sewage becomes quite a problem, and several States have strict regulations for handling this shellfish. In Massachusetts, digging is carried out in the presence of wardens who see that all dead or damaged clams are removed. The clams are then placed in wire boxes, washed with chlorinated water and the boxes of clams are then placed in large tanks of water in which the chlorine content is regulated to maintain it at about 0.5 p. p. m. Every 12 hrs. the water is changed and the clams remain in the tanks at least 24 hrs. or until the count for *Escherichia coli* is less than 140.

3. Crab and Lobster

The principal crab taken on the Atlantic coast is the blue crab, *Callinectes sapidus*, and on the Pacific coast, the Dungeness crab, *Cancer magister.* The chief crab-producing area in the United States is on Chesapeake Bay, although considerable quantities are also produced in Louisiana, Florida, and Oregon. The principal method of capture is by means of trotlines. These are lines up to 2000 ft. or more in length and held to the bottom by weights at each end and marked by buoys. Bait is attached to "snoods," short lines 6 to 8 in. long fastened at intervals to the main lines. Dredges, somewhat similar to those employed in the oyster industry, are also used, and in some cases a modified form known as a scrape is employed in which there are no teeth on the front bar. This gear is used extensively for soft crabs, *i. e.*, those which are about to molt. Soft crabs are taken during the summer months and hard crabs during the winter season.

A large portion of the crab is marketed as cooked, picked crab meat. The crabs are steamed, the meat picked by hand, and packed in friction-top tin cans (or, since the wartime tin shortage, in waxed cardboard containers). The containers are then shipped in boxes or barrels of crushed ice. Recently the practice of pasteurizing the crab meat as devised by Anzulovic and Reedy[21] has been adopted. The meat is placed in $^1/_2$-lb. or 1-lb. C enameled tin cans, the cans sealed and then placed in baskets in a retort or other device whereby the temperature can be controlled, and the cans heated for 1 min. at 170° F. The crab meat remains in the sealed can until used by the consumer. This process kills many microorganisms which are picked up during the packing process including *Escherichia coli.* The crab meat is still perishable but keeps much better than that not pasteurized. If kept at temperatures below 43° F., such pasteurized crab meat remains in good condition for at least 6 weeks.

In past years the Japanese have put up large packs of canned king crab, a part of which was taken from waters adjacent to Alaska and packed on floating canneries. American production in this area has been hampered by lack of knowledge as to areas of abundance, methods of taking, and other factors. A comprehensive survey[22] has recently been made as to the possibility of developing an American fishery for king crab in Alaska. Sufficient information was obtained to indicate that a crab fishery of considerable magnitude could be developed in this area, and it is believed that at the end of the war such operations will begin on a large scale.

Lobsters are caught principally in the New England States and the Maritime Provinces of Canada and Newfoundland. Pots consisting of boxes made from strips of lath or other material and containing a funnel-shaped opening through which the lobster enters but cannot find its way out are used as gear. Pots are usually 4 ft.

---

[21] J. V. Anzulovic and R. J. Reedy, *Fishery Market News*, 4 (No. 1), 3; (No. 2), 9 (1942).

[22] R. W. Harrison *et al.*, *Fishery Market News*, 4 (No. 4, Supplement), 3 (1942).

long, 2 ft. wide, and $1^1/_2$ ft. deep. Since lobsters are usually sold alive, it is necessary to keep them in sea water as long as possible. After removal from the pots it is customary to place them in "live-cars" until the entire catch is sold. These are rectangular-shaped boxes with openings through which sea water can enter and they are moored near shore. Live lobsters are shipped packed in ice in barrels or boxes. Shipment by airplane has become common within recent years.

4. Shrimp

Shrimp are caught along the Atlantic and Gulf of Mexico coast from North Carolina to Texas with principal fisheries centering at Louisiana. Small catches are also taken in California and Alaska. Fishing is carried on by means of the otter trawl and in waters within about 10 miles of shore. About half the catch is canned, about one-third marketed fresh, and the remainder frozen, dried, or otherwise preserved.

Part of the shrimp received at the canneries has been iced on board the fishing vessel, and that which has not been so treated is cooled in ice to facilitate picking. The meat is picked from the shell by hand and the white meat so obtained is blanched by heating in 10% brine for about 6 min. for wet-packed shrimp or 9 min. for the dry-packed product. The blanching process brings out the characteristic red color. The meat is next graded according to size and placed in the cans. Hot 2.5% brine is then added to the wet-packed cans, the cans sealed, and processed in vertical retorts.

Shrimp for the fresh market is prepared by removing the head, and it is packed in barrels or boxes of ice and marketed largely in the South Atlantic and Gulf States.

XII. BIBLIOGRAPHY OF FISHERY TECHNOLOGY

Owing to space limitation it has been impossible to cover the field of fishery technology as thoroughly as might be desired. Since sources of original literature references in this field are not widely known, a brief discussion of such sources together with mention of a number of papers and reports which give fairly complete reviews of certain phases of the field are given. No attempt is made to present anything like a complete bibliography. Only papers which give a fairly comprehensive review of some rather general topic are included and a number of phases of fishery technology where no such reviews are known to exist, are not treated at all.

A large part of the research on fish and fishery products is carried out by governmental agencies. While considerable research is undertaken by laboratories of some of the larger commerical fishery concerns, much of their results and findings is not available to the general public. In the United States, the Fish and Wildlife Service, Division of Fishery Industries, of the U. S. Department of the Interior carries out research on fishery technology in its four fishery technological laboratories located at College Park, Md., Seattle, Wash., Ketchikan, Alaska, and Mayagüez, Puerto Rico. The last two laboratories are operated jointly under the territory of Alaska and Puerto Rico, respectively. Progress reports of the work carried out by these laboratories appear in the monthly periodical, *Fishery Market News* and in the annual bulletin, *Fishery Industries of the United States*. The latter also contains comprehensive statistics on the landing of fish in the United States and Alaska. Progress reports of the Fishery Products Laboratory at Ketchikan, Alaska, are also issued at irregular intervals by the Fishery Experimental Commission of Alaska. Completed projects are reported in Research Reports (formerly Investigational Reports) or sometimes as various special memoranda. Many papers are also published in various scientific and trade journals.

In Canada research on fishery technology is carried out by three Fisheries Experimental Station Laboratories of the Fisheries Research Board of Canada located at Halifax, N. S., Grand River, Quebec, and Vancouver, B. C. (formerly Prince Rupert, B. C.). Progress reports of investigations under way are issued in two series, that of the first two named laboratories in one case and that for the Vancouver Station in the other. Most completed reports are published in the *Journal of the Fisheries Research Board of Canada* and its predecessor journals (*Journal of the Biological Board of Canada* and *Contributions to Canadian Biology and Fisheries*).

In Great Britain fishery technological research is carried out at the Torry Research Station of the Department of Scientific and Industrial Research at Aberdeen. Brief summaries of experimental work are published in the Annual Report of the Food Investigation Board and completed investigations are written as Special Reports or as papers in scientific and technical journals.

Much information on current developments in the fisheries is to be found in the yearbooks issued by *Fishing Gazette* and *Pacific Fisherman*.

The most comprehensive source of information on fishery technology is Tressler's *Marine Products of Commerce* (1). This 762-page book contains detailed descriptions of the fisheries, methods of preservation, fishing gear, and by-products, and completely covers the whole field of fishery technology. Inasmuch as this book was published in 1923, much of the information is now out of date.

Loeffler (2) presents a very comprehensive literature review of fish spoilage and methods of detecting it. Griffiths (3) gives a review of the bacteriology of fresh fish. In a report (4) on handling of mackerel considerable information on spoilage of this species which is applicable to spoilage of fish in general is included. A thorough discussion of organoleptic tests for fish is given by Anderson (5) and although this paper was written twenty-five years ago it is still the best source of material on this subject.

A fairly complete bulletin on fish refrigeration has been written by Taylor (6). More recent developments in refrigeration of fish are given in a review by Lemon (7). A booklet by Zarotschenzeff (8) contains considerable information on freezing and cold storage of fish from both a technical and a scientific standpoint.

Tressler (9) and Taylor (10) have written fairly comprehensive bulletins on salting of fish.

By far the best source of information on fish oils is that contained in Bulletin No. 59 of the Fisheries Research Board of Canada (11). This 442-page bulletin covers every phase of marine animal oils in a very comprehensive way. A description of the fishery by-products industry is given in an article by Lovern (12). A bulletin by Harrison (13) gives a very complete description of the menhaden industry which produces a large share of the total fish oil and meal manufactured in the United States.

(1) Tressler, D. K., *Marine Products of Commerce.* Chemical Catalog Co., New York, 1923.

(2) Loeffler, H. J., "Quantitative Estimation of Incipient Decomposition in Canned Salmon." Ph.D. Thesis, Stanford University, 1938.

(3) Griffiths, F. P., "A Review of the Bacteriology of Fresh Marine Fishery Products," *Food Research,* **2,** 121 (1937).

(4) Stansby, M. E., and Lemon, J. M., U. S. Fish and Wildlife Service, *Investigational Rept.* **1** (1941).

(5) Anderson, A. G., "On the Decomposition of Fish," *Report Fishery Research Board Scotland,* **26** (Part 3), 13 (1907).

(6) Taylor, H. F., "Refrigeration of Fish," U. S. Bur. Fisheries, *Document* **1016,** 501 (1927).

(7) Lemon, J. M., "Developments in Refrigeration of Fish in the United States," U. S. Bur. Fisheries, *Investigational Rept.* **16** (1932).

(8) Zarotschenzeff, M. T., "Between Two Oceans: Rapid Chilling and Freezing Systems for Fish and Meat," *Cold Storage and Produce Rev*, **33,** 1 (1930).

(9) Tressler, D. K., "Some Considerations Concerning the Salting of Fish," U. S. Bur. Fisheries, *Document* **884,** 1 (1920).

(10) Taylor, H. F., "Principles Involved in Preservation of Fish by Salt," U. S. Bur. Fisheries, *Document* **919,** 1 (1922).

(11) Brocklesby, H. N. (editor), "Chemistry and Technology of Marine Animal Oils with Particular Reference to Those of Canada," Fishery Research Board of Canada, *Bull.* **59** (1941).

(12) Lovern, A., "The By-Products of the Fishing Industry," *Chemistry & Industry,* **15,** 75 (1937).

(13) Harrison, R. W., "The Menhaden Industry," U. S. Bur. Fisheries, *Investigational Rept.* **1,** 1 (1931).

Chapter XV

POULTRY AND EGGS

M. E. Pennington

New York, N. Y.

I. POULTRY

Still to be heard in India is the crowing of the jungle cock, the *Gallus bankiva*, from which descended the fowls of the ancients of Persia, Egypt, Greece, and Rome. These birds were common in western Europe when voyagers and colonists were making their way to the New World. Of course they were brought over, because the chicken is a home bird and was, even when he originated in the jungle, a source of food. The turkey, on the other hand, is an American bird and was found here by early explorers who took hens and toms to Europe when they returned. Side by side with man through the ages we find the cock and hen figuring first as timekeeper and watchdog—and so linked to the unseen powers of the universe, and later as a producer of food for the family. From such humble beginnings has our "billion dollar" poultry industry developed.

1. Size of Industry

Poultry and eggs have long been referred to as the "billion dollar industry." Excluding all other poultry—and the value of turkeys, ducks, and geese is large—chickens and eggs brought to the farms in 1937 (the year of last census) a gross return of approximately $1,000,000,000. The total returns in farm income in that year for all products was about $8,000,000,000.

During 1937 about 3,000,000,000 dozen eggs were produced on United States farms, of which about 2,300,000,000 dozens were sold for food and for commercial hatcheries. These added about $500,000,000 to the farm cash income. During the same year our farms produced about 650,000,000 chickens, of which about 384,000,000 were sold—adding $244,000,000 to the cash farm income.

2. Geographical Distribution

Rare indeed is the farm which does not raise a few chickens. But the corn belt—from its northern reaches to the Mexican border—accounts for about half of our total production of eggs, most of which come from farm flocks. Other regions, such as the North Atlantic Coast States and the Pacific Coast States, have concentrated commercialized production in certain areas. The specialized hennery is becoming more and more prevalent—and the number of laying hens per unit is increasing.

In line with commercial henneries for egg production, we have "broiler factories," where birds are produced for meat—cockerels and pullets alike finding their way to the dressed poultry market. The bulk of this industry is along the Middle Atlantic Seaboard, although Arkansas has long raised broilers on a commercial scale. Now smaller units, comprising 2000 to 3000 birds, are appearing here and there in the Middle West. Twenty thousand birds to a unit is not unusual in Maryland and Delaware. The total production of the southeastern sections of Delaware and Maryland and contiguous portions of Virginia was estimated to be about 180,000,000 lbs. in 1941.

The rapidity with which the tonnage of poultry and eggs on farms can be increased is a valuable asset in wartime. We look to hogs and hens for a quick supply of protein food. The statistics already compiled for 1942 are of astronomical proportions.

The year 1942 began with 368,000,000 laying hens on farms. Augmented by late hatches of pullets, they produced in the first seven months of the year 3,050,000,000 dozen eggs. The quota set by the United States Department of Agriculture for 1942 was 4,500,000,000 dozen.

Between January and August, 1942, the hatcheries had turned out 1,062,578,000 chicks—presumably 50% were pullets, many of them from early settings and therefore in egg production by early autumn. The cockerels went to market as roasting chickens—millions of pounds of them, but still not enough adequately to supplement the supply of beef and mutton. Accordingly, the Secretary of Agriculture, on September 15, 1942, asked the industry to produce 200,000,000 additional chickens for meat purposes, saying, "200,000,000 additional chickens marketed at not less than three pounds each would produce at least 600,000,000 lbs. of additional meat for consumers this winter. This would help to maintain civilian supplies and still allow for necessary limitation of civilian consumption of other meats to make sure that ample quantities are available for our armed forces and those of our allies."

To produce millions of pounds of meat at will within a few months and regardless of the season is a striking illustration of the great advance in every branch of the poultry industry as compared with even 50 years ago when hens laid a clutch of eggs in the spring, hatched and mothered a setting, and called the year's job done.

3. Turkeys

The settlers in the virgin land of America found wild turkeys in abundance from Maine to Mexico. The hunters brought them in with quail, pheasants, prairie hens, and passenger pigeons. But as the farms and people multiplied, these birds were more and more restricted in their habitat and as the supply of wild birds lessened, the farmers supplemented it with domesticated turkeys. Large flocks were common in New England and later in Kentucky, Tennessee, and Texas.

But flocks of chickens on the farms also increased and the clean land ranges grew smaller, so that turkeys and chickens were intermingled. The turkeys began to be subject to a serious disease known as blackhead—so serious that flocks grew smaller and smaller until on many farms there were no turkeys.

A pathologist, Dr. Theobald Smith, found that blackhead was caused by a bacterial infection and that chickens were carriers of the organism. He published his findings and said that turkeys would live and thrive if raised on land which had not been contaminated by chicken droppings. It was more than 25 years later that the practical application of Smith's findings again brought turkeys to the farms. They are now recognized as a highly specialized crop and are carefully kept away from chickens.

Turkey raising is a great industry. In 1941, over 33,000,000 birds were raised. Egg-producing flocks in certain sections of the country furnish eggs to hatcheries making a specialty of turkeys. The poults are shipped long distances to be housed with infinite care in brooders and later aired and sunned on wire runways, with never a chance to pick a crumb of soil that might carry blackhead with it. Still later the young birds may be turned out on ranges sown to grass or alfalfa, but fed, watered, and sheltered until ready for market.

Such culture has made turkeys almost a year-around product, though they are still looked upon as cold weather eating and still grace our tables at Thanksgiving and Christmas.

4. The Flesh of the Common Fowl

Poultry for food purposes today is a far cry from the haphazard, mongrel hen and her family strolling along the roadside or busily scratching in the farm barnyard, such as so often characterized the industry 50 years ago. Such hens are even yet seen here and there in farm communities. Many factors have entered into the change, chiefly the increase in the cash value of the hen and her eggs, not to mention the demand for her sons from the time they are broiling delicacies until, soft meated and heavy, they form the basis of the home dinner.

There has been a sharp differentiation between breeds for egg production and breeds for the production of meat. This distinction is, however, growing less. Henneries, especially along the Pacific coast and the Atlantic coast south of New England, have specialized in Mediterranean breeds producing eggs with white shells; while New Englanders and some Midwesterners have used utility breeds, such as the Plymouth Rocks and Rhode Island Reds, which lay eggs with brown shells. The poultry breeders have been working to increase the size and meat quality of Leghorn chickens and they have been equally busy breeding to increase the number of eggs laid by Utilities. Hence, we now have Leghorn roasters weighing four pounds and over, and utility hens which match Leghorns in egg production. However, we still denote Leghorns as egg producers and the Utilities as meat stock birds.

But well-bred or pure-bred poultry is not the only requirement if a chicken is to be satisfactory for food or as a money maker. It must be adequately and properly fleshed and then it must be expertly killed, picked, cooled, graded, packed, and, usually, transported to market. Each of these operations will be dicussed in its appropriate place. Meanwhile, let us see of what these chickens are composed.

Table 93 gives the edible and inedible portions of chickens[1] just as they are marketed by the farm—but we know well that some farmers have well-fleshed birds while others do not adequately feed their flocks. The wide variation in their composition is indicated in Table 94, where the differences between maxima and minima are outstanding.

TABLE 93

GROSS COMPOSITION OF YOUNG CHICKENS[a]—BARRED PLYMOUTH ROCKS

No. of birds	Total edible portion and crude gizzard fat, per cent of chilled weight						Bones, %	Offal, %
	Total edible portion				Crude gizzard fat	Total		
	Meat	Skin	Edible viscera	Total				
78	39.0	7.4	7.3	53.7	1.5	55.2	17.7	27.1

[a] M. E. Pennington, H. A. McAleer, and A. D. Greenlee, U. S. Dept. Agr., *Bull.* **657** (May, 1918).

To give the consumer as much meat as possible and to get as uniform a pack as possible, the poultry dressers feed the birds for from 3 days to 2 weeks in feeding stations. These stations are large, airy buildings equipped with wire cages in tiers four high, about ten birds to a compartment, with feed troughs hanging outside the cages to keep the feed and water clean. Here the "chickens," all of which are young enough for the growth impulse to help in the quick gains in weight, put on more flesh and also improve the quality by introducing fat into the muscle in the place of water. Hens, even if they do not gain much weight, have a softer, better flesh and smoother skins after feeding. Table 95 gives the results of such feeding at the packing house.[2]

TABLE 94

GROSS CHEMICAL COMPOSITION OF FLESH OF COMMON FOWL[a]

	Water, %	Fat, %	Ash, %	Protein, %	Nitrogen, %
Average of 32	65.33	13.99	0.89	19.79	3.298
Standard of deviation	4.10	4.99	0.05	1.59	0.507
Maximum	71.81	28.92	0.98	22.33	3.528
Minimum	54.23	6.35	0.77	16.08	2.981

[a] W. A. Maw, *Can. J. Research,* **11,** 613 (1934).

As the figures indicate, the edible portion, as the birds come from the farm, is much higher in the mature birds than in young chickens. While the trend is the same after fleshing, the differences are less marked. Range hens have 14.52% more edible material than broilers, while after feeding the difference has decreased to 11.30%. But always there is more edible material and less waste in the mature than in the young bird.

---

[1] "Chickens" are birds of the hatch of the current year, not sexually mature and are classed as "broilers"—under 3 lbs., "fryers"—3 lbs., and "roasters"—3 lbs. up. "Fowls" are hens which have laid eggs. Mature roosters are "cocks."

[2] J. S. Hepburn, A. W. Broomell, A. D. Greenlee *et al.,* U. S. Dept. Agr., Bur. Chem., *Bull.* **1052** (March, 1922).

Fleshing changes the composition of the edible portion of the birds as shown in Table 96. Fat takes the place of water in the tissues and the relative amount of protein is also reduced. The caloric value goes up as well as the palatability, since the latter is very dependent upon the amount of fat and its distribution through the tissues.

TABLE 95

DISTRIBUTION OF WEIGHT IN RANGE AND FED BIRDS

Class	No. of birds	Percentage chilled weight								
		Meat	Skin	Crude gizzard fat	Edible organs	Eggs	Total edible	Offal	Bones	Total inedible
Range										
Broilers	114	40.39	7.54	1.31	6.82	.	56.06	21.19	17.75	43.94
Springs	12	45.92	8.81	5.95	1.93	.	62.61	21.65	15.76	37.41
Roasters	18	44.05	9.48	5.68	3.00	..	62.21	21.62	16.15	37.77
Hens	48	38.87	14.68	9.87	4.80	2.38	70.59	18.32	11.10	29.42
Fed										
Broilers	56	39.00	10.41	4.21	6.53	..	60.15	23.81	16.10	39.91
Springs	8	43.10	13.15	5.53	5.29	..	67.07	19.96	12.98	32.94
Roasters	8	40.60	12.61	5.81	5.44	..	64.46	20.48	15.10	35.58
Hens	32	37.93	14.67	10.50	5.21	3.09	71.40	18.43	10.18	28.61

TABLE 96

COMPOSITION TOTAL EDIBLE PORTION OF CHICKENS BEFORE AND AFTER FEEDING[a]

Class	Total edible portion, %					
	Water		Fat		Protein	
	Before feeding	After feeding	Before feeding	After feeding	Before feeding	After feeding
Broilers	70.87	63.39	7.23	16.91	20.11	17.44
Springs (fryers)	68.07	62.06	12.03	20.26	18.50	17.22
Roasters	64.96	61.60	16.55	21.90	17.33	14.42
Hens	51.95	50.67	29.26	32.18	17.00	14.94

[a] Hepburn, Broomell, Greenlee *et al.*, U. S. Dept. Agr., Bur. Chem., *Bull.* 1052 (March, 1922).

The market grades of dressed poultry show a definite relation to the amount of edible meat and fat in the carcass. If grades of roasters, for example, be designated as A, B, and C, there is observed a close relationship between the analysis of the dressed carcass and the grade in that the meat of the lower grades is drier when cooked and there is a greater loss of weight during cooking. The A and B grades lost more fat in cooking than the C grade, but still the meat was more palatable. The actual amount of meat and fat warrants a higher price for the better grades.[3]

Not only does the actual quality of poultry follow the commercial grading but the detailed analysis of the protein of broilers, roasters, and fowls gives a clue to the reason for the additional tenderness and flavor of the young chicken. There is a greater amount of noncoagulable nitrogen in the broilers than in the roaster and the least of all in the old fowl. Furthermore, the amount of soluble protein is greater in the young than in the old bird.

---

[3] W. A. Maw, R. Holcomb, E. E. Rodger, and A. M. Franklin, *U. S. Egg Poultry Mag.*, **42**, 276, 314 (1936).

Osborne[4] included among his classic studies of hydrolyzed proteins the flesh of mature hens and found the following amino acids and amines:

	Per cent		Per cent
Glycine	0.68	Glutamic acid	16.48
Alanine	2.28	Tyrosine	2.16
Leucine	11.19	Arginine	6.50
Proline	4.74	Histidine	2.47
Phenylalanine	3.53	Lysine	7.24
Aspartic acid	3.21	NH_3	1.67

He made the interesting observation that, except for the high proportion of lysine, 7.24%, the results were very similar to those obtained from proteins of leguminous seeds.

Chicken fat, according to Pritzker and Jungkunz,[5] gives analytical figures close to lard, but the iodine number is higher. They found no solid, unsaturated acid. In this they agree with Hilditch, Jones, and Rhead[6] who obtained no evidence of acids more unsaturated than linoleic in the C_{18} group, to which about 65% of the component acids belong. These authors state that chicken fats possess specific characteristics which distinguish them from the fats of quadrupeds and also from fat of fishes and marine animals. They fall into the broad group of deposited fats which are found in non-aquatic animals in which the component fatty acids contain only about 30 to 35% of saturated acids. They are not of the saturated type found in cattle, hogs, and sheep.

The chicken, however, appears to modify the body fat to a certain extent depending upon the type of fat in the food. Thirty or more years ago the poultry industry in the United States attempted to force-feed chickens according to the English method, using beef and mutton tallow mixed with cereal grains, largely maize. The body fat, especially that around the gizzard, became firm in consistency and light in color, and the flavor of the chicken was quite different. This practice was soon discontinued in favor of feeding cereals, especially wheat and oat flour with buttermilk.

These observations received confirmation in the work of Cruickshank[7] who fed palm and other oils and fats and observed very definite changes in the color and consistency of the body fats of the birds.

In common with that of other animals, the fat of poultry contains enzymes which, after death, break it down. The most common enzyme is lipase, which splits the fat, increasing its acidity. Since the fat removed from the carcass immediately after killing is almost neutral, it is probable that the lipase is present as zymogen from which the enzyme is liberated after death. As the fat ages, the acidity increases—a fact which has been utilized to determine the freshness of market birds.[8] In addition the crude fat of chickens always contains catalase, peroxidase, oxidase, and, in most cases, reductase.[9]

---

[4] T. B. Osborne and F. W. Heyl, *Am. J. Physiol.*, **22**, 433 (1908).

[5] J. Pritzker and R. Jungkunz, *Chem. Abstracts*, **26**, 4387 (1934).

[6] T. P. Hilditch, E. C. Jones, and A. J. Rhead, *Biochem. J.*, **28**, 786 (1934).

[7] E. M. Cruickshank, *Chem. Abstracts*, **28**, 6790 (1934); *ibid.*, **29**, 7404 (1935).

II. DRESSING POULTRY IN THE PACKING PLANT

It is plainly evident that the high-quality protein of the flesh of the common fowl and the delicacy of its fat call for specialized and accurate treatment in its killing and preparation for market. The modern poultry packer with his feeding station, his coolers and sharp freezers, his expert graders and packers is equipped to utilize the scientific findings which have come from the technological laboratories and experiment stations. The result is a product which can be shipped anywhere in the world, provided refrigeration is available, and will keep in excellent condition for more than 9 months.

1. Bleeding and Braining

In common with all meat animals the blood must be drained out of the carcass if appearance, flavor, and keeping are to be satisactory. The time-honored farm procedure involving a block, a hatchet, and a fairly strong arm has been discarded. The approved method is to sever the jugular vein after piercing the brain in the region of the *medulla oblongata*. This paralyzes the muscles, especially those controlling the feathers. The bird then bleeds readily and if the cut across the vessels is accurately placed, the carcass is drained of blood.

The cutting of the veins can be made inside the head at the back of the throat. Or, a cut is made outside the head at the base of the bill wide enough to sever the two veins, one of which runs down each side of the neck.

Instead of puncturing the brain, the use of an electrical shock to stun the birds is growing in favor. Various devices are employed for this purpose. The current should be adjusted to the size of the bird to be stunned, else picking will be difficult.

2. Picking

As soon as the veins are cut and the brain is pierced, removal of the feathers begins. The "rougher" grasps a big handful of tail feathers and pulls them out. Then he pulls the heavy wing feathers and, if the bird is in full feather, a few handfuls over the body are removed. The ease with which these feathers come off depends upon the accuracy of the "stick"—that is, the accurate destruction of brain tissue.

In past years the birds were "dry picked," that is, all the feathers were removed by hand, girls generally doing the work after the rougher had pulled the heavy quills. Dry-picked birds are most resistant to decomposition, but they are seldom free from tiny feathers and the "pins" which are about to break through the skin, and so are less attractive to the customer. For this reason the "semiscald" process is almost universally used nowadays. After roughing, the birds are immersed in warm water heated at 128-132° F. for approximately 30 sec., then picked either by hand or by machine. If the killing is properly done and if the temperature of the water is correct, the feathers are easily and cleanly removed.

---

[8] M. E. Pennington and J. S. Hepburn, *J. Am. Chem. Soc.*, **34**, 210 (1912).

[9] U. S. Dept. Agr., Bur. Chem., *Circ.* **103**, 6 (1912).

This semiscald must not be confused with the outmoded method of dipping into water heated to 190° F. or over. While "hard scalding" does make feather removal simple, it shrinks the skin, changes its color, alters the composition of the subcutaneous fat, and seriously interferes with the keeping quality. Hard-scalded poultry is seldom seen these days and then only in the local markets of comparatively few towns and cities, especially in the Midwest.

Fig 46.—"Stripping off the coating of wax removes the feathers." (Figs 46 to 50 are reproduced by courtesy of the Institute of American Poultry Industries.)

The increasing cost of poultry packing house labor and its scarcity has stimulated the invention of machines for removing feathers and also methods for removal of the small feathers which are time-consuming even though the birds have received a semiscald.

What is known as "wax picking" has become very popular—and recently it has been combined with a rubber finger picking machine, by which the dressing of the bird is expedited, the cost is lowered, and excellent results are obtained. After the semiscald the birds are run through the picking machines which remove most of the body feathers. Then they are dipped into a melted wax of special composition and freezing point to form a thin, even coating over the entire carcass. The wax which sets quickly, is flexible and when stripped off takes with it the remaining feathers and pins without tearing or scarring the skin as so frequently happens in hand picking.

3. Cooling

Removal of animal heat is one of the most important operations in the preparation of carcasses for marketing. Not only is flavor influenced by the rate and the thoroughness of the removal of animal heat, but the length of the marketing life of the animal is largely determined thereby.

The beef packers of the corn belt were among the first to utilize mechanical refrigeration to cool the slaughtered animals uniformly and the year around. When the poultry industry of the corn belt grew to such a size that it was as logical to ship dressed poultry eastward as to ship dressed steers or hogs, the packers cooled the dressed poultry in the same fashion. The birds, immediately after picking, were hung in refrigerated rooms which maintained temperatures between 32 and 38° F. for about 24 hrs. by which time the entire body of the bird was at the temperature of the cooler.

But cattle were driven or shipped in large numbers to market centers such as Omaha and Chicago, where packing plants, built at great expense, awaited them. The raising of cattle for food was a recognized, organized industry. Poultry, on the other hand, was a side line on almost every farm, looked after largely by the farm women and sold by them to the poultry buyers in near-by towns. What was the poultry buyer to do with his purchases? It would not be profitable to ship to the few large packing plants for many a bird would die en route. So poultry packing plants were established in towns, the birds dressed there, and shipped to markets large and small. Most of these packing plants were small enterprises. Many of them did not ship a carload--that is, 20,000 lbs.—per week.

Mechanical refrigeration 50 years ago was a relatively new field and the machines were ill adapted to the uses of such small plants as those dressing poultry. But ice was readily available, especially sufficiently far north so that dependence could be placed upon ice naturally frozen. If poultry was killed and picked and then plunged into ice water and later packed in finely chopped ice, the temperature within the body cavity could be reduced to approximately the temperature of ice. So cooled and packed in barrels with ice the birds were shipped by freight and express to Atlantic Seaboard markets. With an abundance of ice, great care in operating, and prompt transportation, their condition upon arrival was good.

But frequently these small shippers were but ill acquainted with the ways of bacteria, the ice in the barrels would melt or the removal of the animal heat would be slow, and then the goods would arrive "slimy" or "green struck" or so nearly off condition that there was no time to put it through the market. Money losses were heavy, there was eternal wrangling with railway claim agents for damages ascribed, rightly or wrongly, to transportation conditions and, worst of all, there was an enormous loss of high-grade food. However, when poultry was killed, cooled in the late autumn or winter in frosty air, packed dry, and shipped in refrigerator cars, its condition was almost invariably good. Why not make one's own wintertime by mechanical cooling?

Of course the experiment was tried. Of course it was a success. Gradually throughout the corn belt the small poultry packers insulated a room or two, put in a small refrigerating machine, dry-cooled poultry at 32–38° F., packed it in boxes standardized to 12 birds each, and shipped not only to the Atlantic Seaboard but to the Pacific as well.

A potent reason for the small ice packing poultry packer staying small was the heavy losses due to spoilage. With the advance in the art of cold air cooling, these losses decreased until now off condition in shipments of dry-packed dressed poultry from the Midwest is the noteworthy exception. The poultry packing plants have increased in size, they are excellently equipped and individually capable of handling many tons of poultry and eggs annually. Moreover, they have become active educators of the farmer in the breeding, feeding, and care of poultry on the farm. Their hatcheries promote better chicks from better eggs. Their trained "service" men advise with the farmer, help him to cull his flock, to feed it most efficiently and economically, and in every way to cooperate with the State experiment stations and county agents to the betterment of the industry.

There is a definite technology for the cooling of poultry, based on the fundamentals of bacteriology and chemistry. The quicker the animal heat is removed, the better, but it is not desirable to cool below the frosting point of the flesh until after the bird is graded and packed in its container. Generally the temperature of the cooler is from 30 to 32° F. when the kill begins. Frosting of the flesh which is indicated by stiffened wings and legs does not begin until below 30° F. The warm birds—which should be rushed from the killing room to the cooler—will soon cause the temperature to rise. There should be sufficient refrigerating capacity to prevent its rise above 38° F. at the end of the kill and the temperature should be down to 32° F. by the following morning—that is, about 12 hrs. after the last warm birds have entered. Racks, specially built, on which to hang the birds by their legs, permit a good circulation of air if not crowded into the room. A moderate forced air circulation can be maintained to speed up cooling if the relative humidity is 94 to 96%, otherwise there will be undue evaporation and the "shrink" will be penalizing—sometimes as high as 1%. For this reason the modern "unit" coolers now so popular among refrigeration engineers are not as satisfactory as pipe coils.

Cooling begins on the surface of the bird and extends gradually to the center of the body cavity. The larger the bird, the longer the time required for cooling. Fat also delays cooling and a long, thin body will cool faster than a short, rotund form. To make sure that cooling is complete the careful packer inserts a thermometer through the vent of the largest bird, pushing the bulb upward until it is practically in the center of the body cavity and so determines exactly the degree of cooling.

Careful studies have been made of the rate of cooling by various investigators, notably Cook,[10] Williams and Funk,[11] and Sweet and Stewart.[12] The aim is to remove the animal heat *quickly*. Cook found that immersing the bird in cold water for 2 hrs. then placing it on a rack in a room at 32° F. reduced the cooling time about 40%. The water absorbed averaged about 2% of the weight of the bird. Stewart has developed a method of speeding up cooling by spraying with a cold calcium chloride brine at 32° F. or less, or water at 33–35° F.—then hanging in a cooler at 30–38° F. Table 97 gives the comparative rate of cooling when the spray method is used.

TABLE 97

COMPARISON OF COOLING METHODS USED ON ROASTERS

No. of birds each trial	Type of cooling	Weight, lbs.		Temp. of cooling med., ° F.	Time in cooling med., min.	Cooling time to 36° F.	
		Range	Av.			range, min.	av., min.
6	Air	5.40–5.96	5.73	34–36	570–690	570–690	640
15	Continuous water spray	4.90–6.66	5.68	33–35	135–240	135–240	200
4	Brine spray	5.20–6.50	6.00	32	40	430–550	520
4	Brine spray	5.10–6.00	5.45	28	40	273–363	308
4	Brine spray	5.10–6.20	5.28	24	40	210–290	280
4	Brine spray	4.50–5.02	4.75	20	25	160–185	180

As shown in Table 97, removal of heat is speeded up by spraying with cold (33° F.) water, and there is a still greater increase in speed when sodium chloride brine at 32° F. is used. Stewart has, therefore, reduced the temperature of the brine to as low as 20° and so has again reduced the cooling time. A comparison of air, water, and brine spray cooling is given in Table 97.

4. Rate of Decomposition in Relation to Temperature

The benefits to be obtained from the quicker removal of animal heat in terms of lengthened keeping time and improved flavor and texture of the market bird are very great. Hanson, Stewart, and Lowe[13] have shown that there was no visible evidence of deterioration in birds held at 35° F. for 18 hrs. After holding 90 hrs. at 35° F. the changes in appearance are noticeable. After 18 hrs. at 35° F. the odor of the kidney is noticeable at the hip joint, but not at the wing joints until after 40 hrs. Eight of the nine birds stored 41 to 43 hrs. at 35° F. scored, after cooking, eight out of a possible ten for flavor of breast muscle, whereas only one bird had thigh muscles scoring eight or higher. The score decreased as the storage time increased.

All experience shows that as the temperature rises the rate of chemical change and bacterial development increases. As previously indicated (see page 528), the acid value of the fat has been used as a measure of the extent of the breakdown. Table 98 gives the acid value of chickens kept for varying periods of time at temperatures of 65 to 75° F. as a maximum and 5 to 10° F. as a minimum.[14]

---

[10] W. H. Cook, *Food Research*, **4**, 245 (1939).

[11] I. L. Williams and E. M. Funk, Univ. of Missouri, College of Agriculture, *Research Bull.* **334**.

[12] M. H. Sweet and G. F. Stewart, *U. S. Egg Poultry Mag.*, **48**, No. 5 (May, 1942).

[13] H. L. Hanson, G. F. Stewart, and B. Lowe, *Food Research*, **7**, 148 (1942).

TABLE 98

THE ACIDITY OF CHICKEN FAT

Chickens kept for 3 days at room temperatures, 65–75° F.:	
Acid value of fresh chicken fat	0.7
Acid value of fat 3 days later	27.0
Chickens kept at temperature of house refrigerator for 5.5 days, 50–55° F.:	
Acid value of fresh chicken fat	0.7
Acid value of fat 5.5 days later	4.4
Chickens kept at temperature of chill room, 32° F.:	
Acid value of fresh chicken fat	0.65
Acid value of fat 15 days later	1.8
Acid value of fat 22.5 days later	3.1
Chickens kept hard frozen for 4, 8, 12, and 16 months, respectively:	
Acid value of fresh chicken fat	0.8
Acid value of fat in storage 4 months	2.5
Acid value of fat in storage 8 months	2.2
Acid value of fat in storage 12 months	2.5
Acid value of fat in storage 16 months	5.4

That long holding, even at temperatures close to 0° F., is accompanied by certain changes in the proteins is evidenced by analytical data presented to the Congress committee.[14] It is given in Table 99.

Presumably these analyses are reported on the wet basis and therefore the increase in total nitrogen may be referred to desiccation. Most striking is the progressive increase in the nitrogen assigned to amino acids. This work was done in 1907–1911, before the significance of "loosely bound nitrogen" as determined by Folin was recognized. Later analyses separated the ammoniacal nitrogen and found it a reliable index of katabolic change in chicken flesh. Another noteworthy change in degradation at the observed temperatures is the decrease in nitrogen insoluble in water. At 65–75° F. the reduction is heavy, less at 50–55° F. and when hard frozen, long holding turns the balance the other way—giving us an increase rather than a decrease in such nitrogen. Contrary to preconceived ideas, the analytical data indicate a greater degradation in the white meat of the chicken than in the dark.

5. Freezing Dressed Poultry

Since the greatest hazard dressed poultry has to face is its perishability, there have been continued and consistent efforts to prevent decomposition when in trade channels. Canning, or sterilizing by heat, has given a stable product but has materially altered the flavor and restricted the forms in which chicken may be served. Freezing, on the other hand, so reduces the rate of chemical change that it is negligible for at least a year and preserves the bird's availability for any type of cookery desired.

---

[14] Testimony by M. E. Pennington, Congressional Hearings, 62nd Congress (1911), "Food Held in Cold Storage," p. 75.

It is obvious that for successful freezing poultry must be well dressed and cooled. The container in which it is to be frozen is also of importance. Not only should the

TABLE 99

DISTRIBUTION OF PROTEIN NITROGEN IN CHICKEN FLESH (EXPRESSED IN PERCENTAGE OF TOTAL NITROGEN)

Sample	Total nitrogen	Total nitrogen in aqueous extract	Coagulable nitrogen in aqueous extract	Albumose nitrogen	Amino acid nitrogen	Peptone nitrogen (by difference)	Nitrogen insoluble in water (by difference)
Chickens held 3 days at room temperatures 65–75° F.:	Light Meat						
Composition of fresh chicken	3.85	25.20	10.48	0.392	11.98	2.25	74.80
Composition 3 days later	3.93	31.40	9.93	1.316	15.47	4.18	68.62
	Dark Meat						
Composition of fresh chicken	3.46	19.19	8.33	0.557	9.50	0.48	80.79
Composition 3 days later	3.41	30.70	10.47	1.55	15.07	3.60	69.29
Chickens held 5.5 days at temperature of house refrigerator 50–55° F.:	Light Meat						
Composition of fresh chicken	3.81	24.90	10.38	0.568	10.71	3.13	75.19
Composition 5.5 days later	3.92	25.45	10.11	0.70	12.49	2.31	74.49
	Dark Meat						
Composition of fresh chicken	3.42	20.64	8.12	0.63	8.53	3.36	79.35
Composition 5.5 days later	3.39	20.09	8.00	0.70	9.82	1.56	77.90
Chickens held at temperature of chill room, 32° F.:	Light Meat						
Composition of fresh chicken	3.92	25.55	11.01	0.625	10.46	3.45	74.44
Composition 15 days later	3.77	23.45	7.98	0.570	10.74	4.15	76.50
Composition 22.5 days later	3.82	27.94	7.64	0.431	11.35	3.49	77.05
	Dark Meat						
Composition of fresh chicken	3.39	20.85	8.34	0.659	8.24	3.62	79.13
Composition 15 days later	3.27	19.26	8.02	0.608	8.11	2.52	80.73
Composition 22.5 days later	3.33	19.41	8.27	0.739	8.78	1.66	80.58

TABLE 99 (*Continued*)

DISTRIBUTION OF PROTEIN NITROGEN IN CHICKEN FLESH (EXPRESSED IN PERCENTAGE OF TOTAL NITROGEN)

Sample	Total nitrogen	Total nitrogen in aqueous extract	Coagulable nitrogen in aqueous extract	Albumose nitrogen	Amino acid nitrogen	Peptone nitrogen (by difference)	Nitrogen insoluble in water (by difference)
Chickens kept hard frozen for 4, 8, 12, and 16 months, respectively:	Light Meat						
Composition of fresh chicken	3.79	24.65	10.52	0.69	9.45	4.02	75.37
Composition in storage 4 months	3.97	24.75	10.70	0.73	8.96	4.37	75.25
Composition in storage 8 months	4.25	24.52	9.70	0.52	10.19	4.01	75.78
Composition in storage 12 months	4.51	22.75	8.30	0.29	12.02	2.13	77.24
Composition in storage 16 months	4.95	23.51	8.91	0.45	12.83	1.32	75.73
	Dark Meat						
Composition of fresh chicken	3.43	20.00	9.16	0.73	8.14	1.94	79.99
Composition in storage 4 months	3.69	20.58	9.79	1.97	7.24	2.54	79.44
Composition in storage 8 months	3.95	21.14	9.19	0.86	8.46	2.85	78.87
Composition in storage 12 months	4.01	19.72	8.32	0.60	9.60	1.24	80.28
Composition in storage 16 months	4.36	19.08	7.84	0.66	9.66	.91	80.86

container protect the birds from mechanical damage, which the commonly used box does, but it should exclude air, which the box does not.

The customary package in the Midwest is a wooden box, frequently wire bound, chambered to hold one dozen birds. Since the number of birds is the unit, the boxes vary from small sizes, which just accommodate one dozen 1-lb. broilers, to those capable of carrying over 60 lbs. of large fowls. The boxes are lined with paper, to which more and more attention is being paid since the evils of air contact are more fully recognized. The paper preferred now is heavily waxed or otherwise treated to restrict air circulation, but so far the efforts in this direction have been only moderately successful. Large, tin containers, which were really tight and absolutely prevented desiccation, were well on their way to acceptance by the poultry industry when the war emergency created the shortage of metals which restricted the use of steel or black plate as well as tin to those food products for which they were essential. There is need for a practical, efficient, airtight package for dressed, noneviscerated poultry.

A few midwest poultry packers still use some barrels in which to ship dressed birds to market, especially fowl and old roosters, and some go to the freezer, but, as we shall see, quick freezing is very important and the complete freezing of a barrel of poultry is a relatively slow procedure. Iced-packed poultry is shipped in barrels but when it must be frozen, it is commonly removed from the barrel and packed in boxes.

(a) *Temperature of Freezing*

For years dressed poultry was "hard chilled" for safety during transportation by holding at a temperature of 10–15° F. for 2 or 3 days. These birds, at the temperature of the usual refrigerator car, arrived at the market with soft, dry flesh and little, if any, evidence of freezing remaining. They could then be marketed as fresh, or be placed in a warehouse sharp freezer for long holding. So treated, dressed poultry almost never arrived at the market in off condition and had ample resistance to withstand the vicissitudes of wholesale and retail market handling.

However, for long-time conservation, freezing should be accomplished as quickly as possible and therefore low temperatures formerly thought commercially impossible are now a matter of common practice. While the tonnage of "quick frozen" poultry, *i. e.*, eviscerated chickens packed singly in packages suited to retail distribution, is constantly growing, the great bulk of dressed poultry going to cold storage is "New York" dressed. Such birds are undrawn and packed twelve to a box in wooden boxes lined with paper.

In response to the demand for rapid freezing, a few warehouses have installed special facilities to that end, but the majority still pile the boxes loosely in their regular poultry holding rooms and, after 3 days to a week, stack the packages as tightly as possible and so hold them.

There are tunnel freezers sponsored by refrigeration engineers which are based on a blast of air at a very low temperature held within a confined space through which the packages are moved on a belt timed so that freezing is completed when the end of the tunnel is reached. The temperature of the air may fall to −35° F. and below. Freezing time, depending on the size and character of the package, may vary from an hour to 5 or 6 hrs. After freezing, the packages are transferred to a holding room at 0° F. or less.

Some warehouses maintain a room at −20° F. or less, in which active air circulation is maintained. Circulating air will freeze poultry in about one-half the time required by air moved only by convection. But rapidly moving air causes desiccation of the product, hence care must be exercised to transfer the packages, as soon as frozen, to a holding room where the minimum of air movement prevails.

We have very specific information indicating that definite rancidity of poultry fat occurs in 3 months when storage temperatures are +15° F. and in 5 months at 10° F., but "no sign of rancidity developed at the end of a year's storage at 0° F."[15] In addition to rancidity of the fat, unpleasant flavors developed when the storage temperatures were above 0° F.[16] The laboratory findings have been confirmed by commercial practices. The warehouses most successfully maintaining quality in frozen poultry are those storing not only at 0° F., but well below that temperature. Probably $-5°$ F. is the most common.

---

[15] D. K. Tressler and C. W. DuBois, N. Y. State Agr. Expt. Station, Geneva, N. Y., *Bull.* 690 (May, 1940).

[16] D. K. Tressler, C. W. DuBois, and F. Fenton, *Refrig. Eng.*, **44**, No. 2 (Aug., 1942).

With good insulation and good operation of freezer rooms it is possible to maintain temperatures of -12 to $-15°$ F. under commercial conditions the year around. In such rooms, poultry can be successfully initially frozen as well as held for long storage.

(*b*) "*Freezer Burn*"

For years the dressed poultry industry discussed the pros and cons of "freezer burn," that is, the formation of light-colored, dry areas of skin or rings around feather papillae, which marred the appearance of the frozen birds. Various explanations were assigned to the condition and so Tressler's[17] paper, stating that desiccation was the cause, was most welcome. Since then considerable work has been done on this subject, notably by Cook,[18] who found that the moisture content of freezer-burned skin was 52.3%, whereas skin from areas not so affected contained 73.4%. Cook summarized his findings by saying that the "rate of evaporation varies directly with the temperature and inversely with the relative humidity."

Loss of "bloom" during storage has also been studied by Cook[19] who states that "the loss of bloom during storage depends mainly on the extent of evaporation. Low temperatures and high humidities tend, therefore, to preserve bloom." Cook found that there was practically no loss of bloom and there was no freezer burn when poultry was stored at $-7.5°$ F. and 100% relative humidity for 83 weeks.

That a tight package accomplishes the same result has been shown by Pennington[20] who packed ducklings in a large metal can, froze and held them at $-10°$ F. At the end of 8 months, the ducklings were free of freezer burn, there was no loss of bloom and no loss of weight. The comparison ducklings in a wooden box and wrapped individually in parchment paper were bleached and burned and had lost 2.5% of their weight.

(*c*) "*Quick*" *Freezing Poultry*

"Quick freezing" as applied to perishable food product is discussed elsewhere in this book. Hence we will consider here only its adpatation to dressed poultry.

As indicated above, the more rapidly poultry is frozen, the better. The original definition of the term "quick frozen" indicated that the object had passed through the zone of maximum water crystal formation in a very short period, usually stated as half an hour. Obviously, it is not possible to freeze a commercially packed box of poultry, even when the temperature is $-50°$ F. and the heat is extracted by conduction, in that space of time. It is possible to reduce the temperature of a single eviscerated 5-lb. roasting chicken from 30 to 20° F. in a Birdseye multiple plate froster in about $1^1/_2$ hrs., but that is a far cry from the freezing of one dozen chickens packed in a wooden box. Almost 3 hrs. are required to freeze a single 5-lb. chicken in an air blast at −25° F. and 5 hrs. at −10° F. with a blast of air. Without an air blast, 10 hrs. are required at −10° F.[21]

---

[17] D. K. Tressler, Library Series No. 12, Institute of American Poultry Industries, Chicago, Ill.

[18] W. H. Cook, *Food Research*, **4**, No. 4 (1939).

[19] W. H. Cook, *Food Research*, **4**, No. 5 (1939).

[20] M. E. Pennington, *Refrig. Eng.*, **41**, No. 3 (1941).

When the birds are packed twelve to a box and when they are not eviscerated, the time required for the temperature in the body cavity of the center bird[22] to pass from 32 to 25° F. is as follows:

Air temp., ° F.	Time required for temperature in center of bird to pass from 32 to 25° F., hrs.
− 3.5	49.5
−18.9	31.0
−35.0	19.6

Small chickens, broilers, freeze in a shorter time. According to Pennington,[20] a wooden box containing twelve little chickens weighing a total of 16 lbs. required 21 hrs. at −10° F. in still air to pass from 32 to 23° F.

Therefore, it would be more accurate to speak of the freezing of poultry as "rapid freezing" rather than "quick freezing," if the original definition of the latter is to hold.

One has only to study the histological picture of chicken muscle rapidly and slowly frozen to see how different are the effects of rates of freezing speed. Such observations have been frequently made beginning with Pennington[23] *et al.*, in 1917 and most recently by Tressler, DuBois, and Fenton.[21] The faster freezing occurs, the less is the change from the unfrozen specimen. As freezing slows down, the water is extruded from the muscle fibers, the dehydrated protein shrinks closer and closer to the cell walls, and the cavities in the tissue increase in size until there is scant resemblance to the original conditions. On thawing, however, and unlike beef, there is very little drip whether the flesh be rapidly or slowly frozen.

6. Evisceration

The removal of the entrails from the body cavity of a chicken is not as simple a matter as the layman might think, and the effects on both the industry and the consumer are many. The retailer or the housewife considers the condition of the entrails as an indication of the presence of/or freedom from disease and consequently its wholesomeness as a food. The odor of the freshly opened body cavity is an indication of the handling which the bird has received since slaughter and whether there has been continuous and sufficiently low temperatures to minimize decomposition. If not, there will be unpleasant flavors in the flesh, especially around the hip joint. The empty body cavity, particularly if washed in a dilute solution of sodium bicarbonate, may be so free from odor as to be misleading and many diseases are diagnosed only by the appearance of the viscera.

---

[21] D. K. Tressler, C. W. DuBois, and F. Fenton, *Refrig. Eng.*, **44**, No. 2 (Aug., 1942).

[22] W. H. Cook and L. Sair, *Proc. Brit. Assoc. Refrig.* (Jan., 1938).

[23] M. E. Pennington, J. S. Hepburn, E. Q. St. John, E. Witmer, *J. Biol. Chem.*, **29**, 33 (1917).

To protect the consumer from these dangers the Federal Government and also certain State boards of health provide poultry dressers with an inspection service in which each bird, after the opening of the body cavity, is examined by a veterinarian. Those showing evidences of disease are discarded. The City of New York will not permit the sale of poultry eviscerated outside the city or state unless it bears the official certificate of such inspection. All canneries doing an interstate business are required to have evisceration under federal inspection.

Sanitation in poultry packing plants where eviscerating is done on a large scale is a matter of rigid inspection and specialized equipment. The work is done on "conveyor tables of sheet metal and channel construction, with automatic cleaning, washing and sterilizing of the conveyor pans or trays during the operation."[24] Giblets are removed with a minimum of tearing, placed on special perforated trays, and washed with a gentle spray of cold water. The carcass is thoroughly washed under sprays of cold water and then wrapped and packaged whole, or, as in the case of fryers and fowl, cut in the usual housefold fashion.

The delicate membranes lining the body cavity of a bird are extremely susceptible to bacterial invasion. Therefore, since it is not possible to eviscerate under aseptic conditions, immediate freezing is generally employed for preservation. Fast freezing is especially desirable. Also, desiccation must be zealously guarded against. Moisture-vaporproof wrappers are commonly used—further protected by water-resistant paperboard boxes. The demand for full-drawn poultry is increasing but slowly, despite the obvious economies to the shipper in transportation and to the retailer in reduced labor in his store. Restaurants, hotels, and institutions requiring large quantities of poultry are gradually learning the advantages of buying in a "ready to cook" form. Wholesale distributors are being asked more frequently to deliver the birds drawn. In order to ensure that the operation be performed in a clean manner and under suitable conditions, boards of health have issued regulations which merchants must follow. The regulation issued by the Board of Health of the City of New York is given below.

REGULATIONS FOR ESTABLISHMENTS ENGAGED IN EVISCERATING POULTRY[25]

REGULATION 115. USE OF CELLAR PROHIBITED.—No establishment engaged in eviscerating poultry shall be located in any cellar.

REGULATION 116. EVISCERATING OF POULTRY: SEPARATE ROOM REQUIRED.—Eviscerating of poultry and processes directly associated therewith shall be conducted in one or more separate rooms which shall be used for no other purpose.

REGULATION 117. THAWING OF FROZEN POULTRY.—Frozen or chilled poultry shall not be thawed n still water. Suitable shallow troughs or tanks with cold, running water and properly installed overflow pipes shall be used for the thawing of poultry.

REGULATION 118. TABLES.—Tables shall be made of smooth, hard, impervious material and shall be so constructed that they can be readily cleaned and inspected, and so placed that all parts are accessible for cleaning and inspection.

---

[24] Harry E. Drews, *Food Industries*, **14**, No. 11, 60 (1942).

[25] Dept. of Health, New York City, Part 7 of Sections 148 and 148A of the Sanitary Code of the City of New York.

Regulation 119. Waste Receptacles.—At each eviscerating table there shall be provided suitable watertight metal receptacles, into which the entrails and other waste from the eviscerating operation shall be immediately placed.

Regulation 120. Method of Eviscerating of Poultry.—Each bird shall be eviscerated in an individual metal tray of seamless construction. Said trays shall be properly washed and sterilized after each use. The eviscerated poultry shall then be cleaned under a suitable spray of clean water and the use of tanks for such cleaning is prohibited.

Regulation 121. Hand Wash Sinks.—A suitable number of hand wash sinks with hot and cold running water, soap, and individual towels shall be provided in the eviscerating rooms.

III. EGGS

The production of eggs in the United States has been briefly touched upon at the beginning of this chapter. The quantities produced have been counted in billions of dozens for many years, yet the increase as a result of wartime requirements has stimulated such a rapid growth that previous figures are quite eclipsed. In 1942 there were produced 3,996,583,000 dozen eggs, an increase of 516,083,000 dozen over 1941, while for 1943 the Secretary of Agriculture has asked for an 8% increase over the volume for 1942. Hatchery men, farmers, and processors of egg products are confidently expected to comply with the request. No other source of high efficiency, animal protein can be increased as rapidly as hens' eggs nor is there any other animal protein food with less inedible material—the shell is about 10% of the total weight of the egg.

1. Composition

Very glibly we say that an egg consists of fats, proteins, mineral salts, and water. We are apt to forget that every essential for the production of a chick, except free oxygen, is contained within the egg shell. In spite of years of study we are still adding to the long list of chemical compounds which an egg contains and still adapting this mixture of substances, so near to living tissues, to uses which permit of no substitution. For example, we use egg as the source of biotin and we grow the vaccine of spotted fever in the embryonic tissues of the developing chick.

To the food technologist, however, it is of importance to know certain gross facts of chemical composition, such as: shell 10.26%, yolk 30.3%, and white 59.35%, as an average of many reported analyses.

(a) *Shell*

The shell is largely composed of calcium carbonate with magnesium and other metals in smaller quantities. The figures usually given are $CaCO_3$, 93.7%; $MgCO_3$, 1.39%; P_2O_5, 0.76%; and organic matter, 4.15%.

There is a very definite structure in the shell of an egg, each layer playing its part in the development of the chick. First, starting from the outside, is a thin coating of a mucin which covers and protects the pores, unbroken when the egg is fresh, but which cracks when the atmosphere dries it out. Under this "cuticle" lies the calcareous layer containing the pores, small holes which may completely traverse the shell and which permit air to reach the developing chick. There may be 6000 to 8000 of these pores when the shell is old and dry, some very small but a few large enough to see with the naked eye and plenty large enough to admit bacterial and mold hyphae when the cuticle has cracked and pulled away from them. Below the chalky, homogeneous layer comes the mammilla layer in which are protruding processes which are large spherulite crystals of calcite with cores of organic matter. This mammilla layer lies just above the shell membrane, that very important structure which plays a large part in the protection of the egg substance.

There are two shell membranes—some say three—composed of chiten and having a well-defined network of fibers interlacing to produce the firm, tough structure with which we are all familiar. The outer membrane is heavier and tougher than the inner which forms the "floor" of the air cell and so is called the egg membrane, though it adheres to the shell when the egg is cracked and the contents dumped out. Bacteria and molds can work their way between the chiten fibers and so get into the interior of the egg, but as long as the outer surface, *i. e.*, the surface lying close against the shell, is dry, penetration is a difficult matter.

(*b*) *Egg White*

The albumin of egg white has long been looked upon as a typical protein. It has all the physical properties which we associate with proteins, and its decomposition products, especially amines and amino acids, indicate its high rank among such nitrogenous substances. It can be prepared in crystalline form and has served as a source of information on proteins in general. In addition to ovalbumin, egg white contains conalbumin, ovomucin, and ovomucoid.

The gross analysis of the white of the hen's egg averages as follows: water, 87.99%; ether extract, 0.02%; ash, 0.65%; protein, 10.44%; total nitrogen, 1.67%; dextrose, 0.5%; carbon dioxide, 1.3 mg. per ml.; *p*H, 8–8.6.

As in all other parts of the egg, the white has a definite structure. If an egg of good quality is broken on a flat plate, there will be observed a thick, opalescent jelly which hugs the yolk and extends well beyond it into the "thin white" which spreads as a thin liquid around the island of thick white. If a cut is made across the thick white, the mass quickly flattens and there is a noticeable increase in the amount of thin white. Indeed, the thick white is a sack with thin white enclosed which, when the wall of the sack is broken, runs out. But some jelly remains—a jelly attributed to a mesh of mucin threads in which the white is held and from which it can be liberated by cutting the threads with a pair of scissors.

(*c*) *Yolk*

Within the white lies the yolk, encased in the vitellin membrane, a very thin and elastic sack bearing upon it the blastoderm which, having been fertilized and afforded appropriate temperature, develops into a chick. Nature with infinite care made it an ideal food for the developing chick. Man finds it an ideal food for himself and especially for his children.

The gross analysis of egg yolk shows it to consist of 33.3% fat, 15.7% protein, 1.1% ash, and 49.5% water.

The chemical composition of egg yolk is very complex. Ovovitellin, ovolivetin, ovolecithin, cholesterol, creatine, creatinine, lactic acid, choline, alcohol, vitamins, enzymes, inorganic salts, xanthophylls, carotene, and fats have been found in the yolk of the hen's egg. We are constantly adding to the list. Not only is the chemical composition complex, but the yolk has a definite structure. Just beneath the blastoderm is a column of white material extending about halfway through the ball of yolk—the latebra. The globules of the emulsion in the white yolk, as this is called, are smaller than those of the yellow yolk. It is the material in the latebra which first nourishes the developing embryo. From this column of white yolk thin layers of similar material extend over the whole sphere, thus dividing it into sections of alternating white and yellow—relatively thick sections of yellow and very thin sections of white yolk. Another evidence of a structure in the yolk is the effect of freezing it *in situ*. At about 21° F. it becomes rubbery in consistency and is quite useless as a food. However, if the structure of the yolk is wrecked, as by breaking the vitellin membrane and by mechanically mixing the liquid, no such denaturing occurs. The yolk will freeze and thaw again with but little physical change, a fact of great usefulness in the utilization of frozen egg for baking and other cookery.

2. Bacteria in Eggs

Generally an egg "rots" because it has become infected with bacteria or molds. Occasionally the hen lays a heavily infected egg but generally, as laid, the egg is sterile or nearly so. Even if bacteria are present in the oviduct and become entangled in the forming white they do not tend to multiply and they may be killed off. There are conflicting findings in the literature concerning the bactericidal properties of egg white. These were largely resolved when Sharp,[26] in 1927, showed that the *p*H in egg white changes and with it the ability to restrict the growth of certain kinds of bacteria.

The *p*H of newly laid eggs is 7.6. Due to the escape of carbon dioxide which is practically complete in 24 hrs. at room temperature, this figure rises to about 9, a *p*H beyond the tolerance of many bacteria. Certain organisms, notably *Bacillus subtilis*, would not grow in egg white at any *p*H tested and we are therefore back to the theory that egg white does possess germicidal properties.

By far the most common route for bacterial infection of eggs in the shell is through the pores of the shell, especially through the large pores which completely traverse all the layers.[27] Mold hyphae can be followed from cuticle to egg substance through a pore by microscopic examination after suitable methods of staining and sectioning.

---

[26] P. F. Sharp and R. Whitaker, *J. Bact.*, **14**, 17 (1927).

[27] L. S. Stuart and E. H. McNally, *U. S. Egg Poultry Mag.*, **49**, No 1, 28 (1943).

Water on the shell, sufficient to enter the pores, is of great advantage to roving bacteria. Studies of eggs, the shells of which have been wet, show a very large percentage infected as compared with eggs which have been kept dry. Obviously, eggs with dirty shells are even more apt to be infected. Obviously, also, if dirty eggs are washed in water, bacteria are carried into the pores, and so more often into the egg substance. For this reason it is better to market or store eggs with dirt on the shells than to wash in water.

Funk[28] has developed a method of washing in a solution of lye which does not further infect the eggs as demonstrated by their keeping in cold storage. It is also possible to demonstrate that dirty eggs washed in solutions which sterilize the shell make for better keeping.

Funk[28a] has also devised a method for pasteurizing shell eggs by heating in oil or in water held at 140° F. He stated that this treatment destroys the bacteria and causes the eggs to retain their desirable physical properties longer than do untreated eggs. It is entirely possible that egg magma can be successfully pasteurized since Epstein reports (*Proc. Dried Egg School*, Kansas City, 1941) that heating sugared yolk at 143° F. for 30 min. reduced the bacteria count from 371,000 per g. to 1500 per g.

That bacteria will penetrate and proliferate in eggs is well illustrated by the relatively large percentage of bacterial and mold rots which develop in dirty eggs in cold storage. Jenkins[29] *et al.*, found after 6 to 11 months' storage at 29–30° F. that 14.4.% of dirty eggs washed directly in water were rotten; dirty eggs wiped with a damp cloth showed 8.8%, while dirty untreated eggs had 6.6%. Clean eggs, not washed, showed only 1.9% inedible eggs.

Since bacteria within the egg are so largely due to infection after laying, it follows that there is not only a great variety of organisms present, but that some of them are of fecal origin since nests are fouled by feces and the hens are frequently soiled ventrally. For some time the presence of *E. coli* was asserted by some investigators and denied by others. Now, however, its presence is generally agreed upon and in some types of rots known as "sour eggs" coliform organisms are in the majority. Some of the salmonellas have also been identified in shell eggs, especially those laid by sick hens. Organisms of the *Pseudomonas* group are found in eggs[30] where they are sometimes responsible for a definite type of rotting. They are of greater significance in that they grow well and fairly rapidly at egg storage temperatures, 29–31° F.; hence when an egg so infected goes into long-hold cold storage it is likely to come out inedible. *B. proteus* and *B. pyocyaneus* were first reported by Artault in 1894 and by various investigators since then. Hadley and Caldwell[31] isolated many species which they grouped into micrococci, motile rods, spore bearers, Gram-negative motile and nonmotile rods and others. Food poisoning due to bacterial decomposition of eggs is discussed by Scott.[32]

---

[28] E. M. Funk, Univ. of Missouri, *Bull.* 277 (Feb., 1938).

[28a] E. M. Funk, Univ. of Missouri, Agricultural Expt. Sta., *Research Bull.* 364 (May, 1943).

[29] M. K. Jenkins, J. S. Hepburn, C. Swan, and C. M. Sherwood, *Ice and Refrig.*, 58, 140 (1920).

[30] R. B. Haines, Food Investigation Board, Low Temperature Research Station, Cambridge, England, *Special Report* 47 (1939).

[31] P. B. Hadley and D. W. Caldwell, Rhode Island Expt. Sta., *Bull.* 164 (1916).

The further we go in the study of the bacterial flora and its source in eggs, however, the stronger the evidence that in the great majority of cases infection takes place after laying and most of the organisms found are ordinary air and water saprophytes. *Achromobacter perolans*[33] is the organism responsible for producing in eggs the odor known as "baker's must." Except for the odor, the egg appears to be in good condition. The odor is characteristic, very offensive and penetrating and persistent, and is a pest in egg-breaking stock. One such egg will ruin a good many pounds of dough. Apparently this organism is transferred from the soil or from wet hay or other soil-contaminated nest material to the egg. It grows well at low temperatures, hence is found in the early spring egg and late in autumn when eggs are coming out of storage.

3. Maintenance of Quality

The food technologist cannot make a bad egg good. He can only take what the hen produces and call science and art to his aid in maintaining that quality. He is materially assisted by the precautions which Nature has taken to guard against such changes in chemical composition and physical make-up as would prevent the development of a chick. The two worst enemies to be overcome are water and heat. A wet shell can readily result in an infection by molds and bacteria. Warmth stimulates cell division in the fertilized egg with resultant chick development; or increased enzyme activity in the unfertilized egg which results in a physical deterioration which lowers palatability and commercial value. Therefore, those who would maintain quality should, from the hen to the table, keep eggs dry and keep them cool. Never wash an egg in water and wipe it with a wet cloth or wet brush unless it is to be used at once. Keep the temperature during marketing well below 60° F. The longer the marketing period, the lower the temperature must be. The accepted temperature is from 29 to 31° F. if cold storage is to hold the egg for 6 months or more. An egg in the shell does not freeze until below 28° F., although out of the shell or in a badly cracked shell, its freezing point is approximately 31° F. On the other hand, a few hours at summer temperatures will so stimulate cell division of the blastoderm in the fertile egg that its commercial value as a high-quality food is seriously impaired.

Much time and effort have been spent in devising practical egg coolers for farm use, especially in the absence of a supply of ice or of mechanical refrigeration. Under such circumstances an "egg storage cellar" can be built and will give protection to the eggs while they are being collected for market. Such a cellar has been designed by the Kansas Agricultural Experiment Station, Manhattan, Kan.

As far as facilities at the concentrating station are concerned, an insulated, mechanically refrigerated cooler is an absolute necessity if quality is to be maintained. Also, in warm weather, eggs must be shipped in refrigerated cars or trucks.

---

[32] W. M. Scott, *Brit. Med. J.*, 56 (1930).

[33] M. P. Spanswick, *Am. J. Pub. Health*, 20, 73 (1930).

4. Candling and Grading

The commercial method of determining the quality of an egg is to hold it before a bright light in a dark room and look through the shell. This process is termed "candling." The "candle" consists of a bright light a 40-watt electric bulb is standard—enclosed in a metal case containing a hole just large enough to permit the illumination of the whole egg. There should be a leather or rubber pad around the hole so that no light escapes when the egg is pressed against the candle. The light bulb should be placed so that its long axis is at right angles to the egg when held blunt end up against the hole.

Fig. 47—Government licensed egg graders candling eggs for packaging under certificates of quality or grade labels as of a particular U. S grade.

The person skilled in determining the condition of the interior of the egg by this method is known as a "candler." An experienced candler can grade from two to five cases per hour depending upon the expertness of the operator, the efficiency of the candle, the condition of the raw material, and the accuracy of grading required. The consumer will not see the egg before the candle. He will see it out of the shell. Hence the candler must translate what he sees into the appearance of the opened egg. The more expert the candler, the more apt he is to break an egg now and then, of which he is not quite sure, and so add to his own knowledge.

One of the most important changes for the candler to identify is evidence of incubation. After keeping for about 5 hours at 100° F. the candler can see a change in the appearance of the yolk and on opening he can detect the thickened area of the blastoderm. Quickly, then, the yolk floats closer to the shell, moves more rapidly, is no longer situated at the equator of the shell, and has a darker appearance. Gradually, too, the "spot" on the yolk, as the candler twirls the egg before the light, grows larger and darker. Finally, when the egg has been kept from 85 to 110° F. for several days, blood vessels form over the germinal area and the elongated aggregation of cells to form the chick is plainly visible either before the candle or out of the shell. Such an egg is classed as inedible and is not, legally, usable for human food.

Other types of deterioration classed as inedible which the candler must recognize are the following:

Black rots, mixed rots, and white rots (addled eggs), eggs with stuck yolks, moldy eggs, embryo chicks, and crusted yolks (long held storage eggs) and bloody eggs. Out of the shell the operator must recognize the egg having a green color in the white or which has a sour odor, since such eggs are heavily infected with bacteria.

The size of the air cell is almost universally used as an index of quality. To a certain extent this is justified, but often more importance attaches to the candlers' conclusions than the facts warrant. The official correlations between air cell depth and grades are given on p. 549 under "Specifications for Official United States Standards for Quality of Individual Shell Eggs."

The "tremulosity" of the air cell membrane has long been a bone of contention. This condition is caused by rough handling of the egg. The junction of the two membranes, continuous when the egg is laid because then the body temperature of the hen has expanded the content, is pulled down as the egg cools, the inner membrane following the egg substance. Hence the "air cell." Rough handling, especially when the large end of the egg is down, causes the vibration of the membrane and so its firm position against the shell membrane is released and it becomes "wavy" or tremulous. This is without effect on the edible quality of the egg.

(*a*) *Out of the Shell*

Commercially we must and do depend upon candling to determine the quality of an egg. But the final word is the appearance out of the shell and as the consumer sees it. It must not be forgotten that eggs vary when they are laid. Frequently an egg will have two yolks. Frequently, also, areas having a translucent appearance, some very tiny—some a quarter of an inch in diameter, are seen on the yolk. These are ascribed to various causes. Schaible, Davidson, and Moore[34] state that the irregularity is due to a break in the continuity of the layers of yolk—white yolk and yellow yolk, the former being less viscid at ordinary temperatures and also much less in quantity. Therefore, if the layer of white yolk is not continuous, the yellow yolk would show as darker spots or irregular areas. "At certain times pressure may be a factor in the natural spotting of yolks, since the yolks of eggs of one individual hen had repeated spotting of a similar design." Occasionally these spots on the yolk can be seen through the shell by the candler, especially if the shells are white and thin. Since he does not know the cause, he is apt to lower the grade of the egg.

Blood may be due to a pinching off of a little blood vessel in the ovary, in which case it adheres to the yolk as a clot. The cause is not pathological, the little clot can readily be removed from the yolk by the consumer, and is significant only psychologically. Quite different is the presence of diffuse blood in the white, due to a hemorrhage in the oviduct, and varying from a pale pink to blood-red. Such an egg is generally heavily infected with bacteria and soon develops an unpleasant odor. A blood clot can generally be seen by candling. Diffuse blood can be seen, even in an egg with a brown shell, if in sufficient quantity. A small amount may be overlooked, especially if the shell is dark brown or thick.

Meat spots, or *liver spots*, as they are sometimes called, are fragments of tissue, red or brown, which float in the egg white. These scraps of tissue are generally gland fragments scraped off the walls of the oviduct by the passage of the egg. They are not pathological and they do not render the egg inedible. They are, except when minute, visible by candling, and eggs containing them are commonly put into a lower grade because of their appearance when opened.

Some hens lay eggs with more thick white and a stiffer thick white than do other hens. Indeed, some hens produce almost no thick white. Their eggs when opened look like stale whites, even though they are just laid. A chart representing gradations of quality in opened eggs has been prepared by A. Van Wagenen of Cornell University. It is reproduced as Figure 48. Egg No. 1 has an abundance of stiff jelly-like white; No. 2, a very fine egg, has a little less jelly and not quite so stiff; No. 3 is more like the "good" eggs in commerce, while from No. 4 on, the appearance coincides with increasing staleness.

This chart represents new-laid eggs only. Had it been prepared from eggs the whites of which had become thin from aging, the yolks would not have stood up as shown in the transverse sections. Yolks tend to flatten with age, an important item in judging quality.

(*b*) *Albumen and Yolk Index*

Some of the knowledge gained by studying the opened egg can be correlated with certain appearances before the candle and so becomes of practical importance in egg grading. The thick, upstanding white and the spherical yolk had long been accepted as evidence of good quality but it was not until these relations were given a mathematical expression that specific use of them was made.

---

[34] P. J. Schaible, J. A. Davidson, and J. M. Moore, *U. S. Egg Poultry Mag.*, **42**, No. 8 (1936).

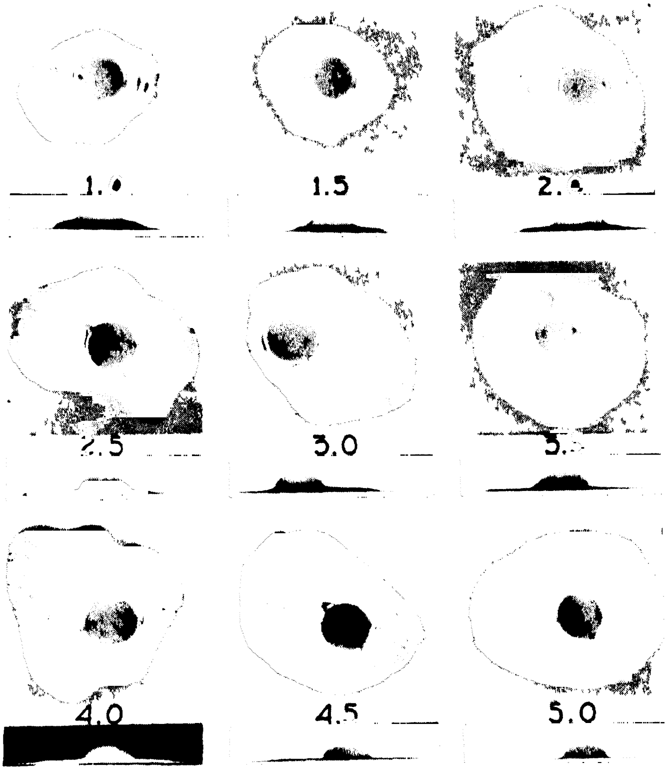


Fig 48.—A photographic study of the various stages of the firm albumen in fresh eggs by Van Wagenen and Wilgus of Cornell University.

Sharp, Stewart, Wilgus, and Van Wagenen at Cornell, and Carver, Wilhelm, Heiman, and others at Washington State College have developed and used in many studies the measurement of the height of the thick white divided by its diameter which gives the "index of the thick white" or the "index of the yolk" depending upon which is being measured.

Parsons and Mink[35] have correlated these indexes with quality as indicated by candling then breaking and matching the appearance of the egg against the Van Wagenen photographic chart with the quality scoring as indicated in the chart. The results are given in Table 100.

---

[35] C H. Parsons and L. D. Mink, *U. S. Egg Poultry Mag.*, **43,** No. 8, 484 (1937).

TABLE 100

AVERAGE RESULTS OF MEASUREMENTS AND EXTREMES OBTAINED ON EGGS ARRANGED IN GROUPS ACCORDING TO SCORE[a]

Groups according to score	Yolk index	Yolk wt., g.	Thick white, %	Albumen index	Area of thick white, sq. cm.	Albumen area index	Albumen area index incl. yolk
Group I—Eggs scoring from 1.0 to 1.5, incl.							
Max.	0.43	22.5	61.3	0.148	58.8	4.52	1.71
Min.	0.36	15.3	41.6	0.053	30.7	1.54	0.79
Ave.	0.39	18.5	52.8	0.078	44.8	2.92	1.32
Group II—eggs scoring from 2.00 to 2.50, incl.							
Max.	0.44	22.2	68.9	0.080	75.9	5.94	2.16
Min.	0.30	14.6	36.0	0.034	37.2	2.50	1.14
Ave.	0.39	19.0	52.1	0.057	55.1	3.68	1.61
Group III—eggs scoring from 3.00 to 3.50, incl.							
Max.	0.43	23.0	69.3	0.075	113.2	8.25	3.37
Min.	0.24	13.2	32.0	0.019	41.3	2.29	1.26
Ave.	0.37	18.4	49.4	0.043	67.3	4.80	2.08
Group IV—eggs scoring from 4.00 to 5.00, incl.							
Max.	0.43	25.4	60.7	0.039	130.0	15.90	4.60
Min.	0.26	14.3	9.5	0.013	50.4	3.59	1.53
Ave.	0.34	18.8	37.8	0.024	81.7	7.49	2.58

[a] From Parsons and Mink, *U. S. Egg Poultry Mag.*, **43** (Aug., 1937).

The commercial grading of eggs, using the candle to see the interior, has come to be an elaborate and, even allowing for the limitations of candling, a fairly accurate procedure. The specifications covering United States grades for individual eggs are reproduced below.

SPECIFICATIONS FOR OFFICIAL UNITED STATES STANDARDS FOR QUALITY OF INDIVIDUAL SHELL EGGS

Effective Jan. 2, 1943

1. U. S. Standards for quality of individual eggs with clean unbroken shells shall be as follows:

U. S. Grade AA. The shell must be clean, unbroken and normal. The air cell must not exceed $^1/_8$ inch in depth and may be regular or slightly wavy. The yolk outline must be free from defects or blemishes visible before the candle. The white must be clear and firm.

U. S. Grade A. The shell must be clean, unbroken and normal. The air cell must not exceed $^2/_8$ inch in depth and may be regular or slightly wavy. The yolk outline may be fairly well defined. The yolk must be practically free from defects or blemishes visible before the candle. The white must be clear and reasonably firm.

U. S. Grade B. The shell must be clean and unbroken, but may be slightly abnormal. The air cell must not exceed $^3/_8$ inch in depth and may show total movement not in excess of $^3/_8$ inch. If the air cell is small (not over $^2/_8$ inch in depth), it may be free. The yolk outline may be well defined. The yolk may show definite but not serious defects visible before the candle. The white must be clear but may be slightly weak.

U. S. Grade C. The shell must be clean and unbroken but may be abnormal. The air cell may be over $^3/_8$ inch in depth, and may be bubbly or free. The yolk may be plainly visible, and appear dark. The yolk may show clearly visible germ development, but no blood due to such development. It may show other defects that do not render the egg inedible. The white may be weak and watery. Small meat spots or blood clots may be present.

2. U. S. Standards for quality of individual eggs with soiled, stained, or dirty shells shall be as follows:

U. S. Light Dirty—Individual egg that has not more than one-eighth of the shell surface slightly stained, slightly soiled, or slightly dirty but without loose adhering dirt and of the interior quality of U. S. Grade B or better shall be classed as U. S. Light Dirty.

U. S. Dirty—Individual egg with more than one-eighth of the shell surface stained, soiled, or dirty, or with less than one-eighth of the shell surface stained, soiled, or dirty to such an extent that it is more than slightly stained, slightly soiled, or slightly dirty, or any egg with slightly stained, slightly soiled, or slightly dirty shell and of the interior quality of U. S. Grade C shall be classed as U. S. Dirty.

3. U. S. Standards for quality of individual eggs with checked or cracked shells shall be as follows:

U. S. Check—Individual egg with either clean or dirty shell that has an open crack or break in the shell but with the shell membrane unbroken and with no leakage of the shell contents shall be classed as U. S. Check.

U. S. Leaker—Individual egg with either clean or dirty shell that has an open crack or break in the shell and shell membrane and with the contents exuding or free to exude through the shell shall be classed as U. S. Leaker.

Explanation of Terms

The Official United States Standards for Quality of Individual Shell Eggs are applicable to eggs that are the product of the domestic chicken hen and are in the shell.

1. **Clean**—A clean shell is one that is free from foreign matter and from stains or discolorations that are readily visible. Eggs with only very small specks or stains may be considered clean, if such eggs are not present in sufficient number in a package to detract appreciably from its appearance. Eggs that show traces of processing oil on the shell are considered clean when classified as "processed," or "shell treated," unless the shell is otherwise soiled.
2. **Light Dirty**—A light dirty shell is one that shows slightly stained or slightly soiled areas that are readily visible but without adhering dirt that could be readily detached and that do not affect more than $^1/_8$ of the shell surface.
3. **Dirty**—A dirty shell is one that has stained, soiled, or dirty spots of considerable size that may affect more than $^1/_8$ of the shell surface, or that has less than $^1/_8$ of the shell surface stained, soiled, or dirty to such an extent that it is more than slightly stained, slightly soiled, or slightly dirty.
4. **Unbroken**—An unbroken shell is one that is free from actual checks or breaks.
5. **Checked or Cracked**—A checked or cracked shell is one that has an actual break in the shell but where the shell membrane is unbroken and there is no exuding of contents from the egg.
6. **Leaker**—A leaker is an egg in which the shell and shell membrane are broken to the extent that the egg contents are exuding or are free to exude through the shell.
7. **Normal**—A normal shell is one that approximates the usual shape and that is of good even texture and strength and free from distinct ridges, rough areas, thin spots, or other conditions not common to good shells. Slight ridges and rough areas that do not affect materially the shape, texture, and strength of the shell are permitted.

8. **Slightly Abnormal**—A slightly abnormal shell is one that may be somewhat unusual in shape or that may be somewhat faulty in texture or strength. It may also show distinct, but not pronounced, ridges, thin spots, or rough areas.
9. **Abnormal**—An abnormal shell is one that may be decidedly misshapen or that may be decidedly faulty in texture or strength or that may show pronounced ridges, rough spots, or other defects.
10. **Depth of Air Cell**—The depth of the air cell, when in its natural position, is the distance from the large end of the egg to the plane passing through the egg at the point where the lower air cell membrane touches the shell.
11. **Regular**—A regular air cell is one that retains a fixed position in the egg and that shows a practically even, smooth outline without any movement when the egg is twirled.
12. **Slightly Wavy**—A slightly wavy air cell is one that retains a practically fixed position in the egg but shows a slight movement, not to exceed $^1/_8$ inch, at any point where its lower shell membrane touches the shell.
13. **Movement not in Excess of $^3/_8$ Inch**—An air cell that shows a total movement not in excess of $^3/_8$ inch from the line where its lower shell membrane touches the shell.
14. **Bubbly Air Cell**—A bubbly air cell is one that has several rather small bubbles within or beneath it, that give it a bubbly appearance.
15. **Free Air Cell**—A free air cell is one that moves freely about in the egg. Such an air cell will seek the uppermost point in the egg, no matter in what position the egg may be held.

Terms Descriptive of the Yolk

16. **Outline Slightly Defined**—A yolk, the outline of which, when viewed before the candle, is indistinctly indicated and tends to blend into the surrounding white.
17. **Outline Fairly Well Defined**—A yolk, the outline of which, when viewed before the candle, is discernible but that has not become definite and distinct.
18. **Outline Well Defined**—A yolk, the outline of which, when viewed before the candle, is quite definite and distinct.
19. **Plainly Visible**—A plainly visible yolk is one that has a definitely discernible outline before the candle and that may appear dark
20. **Free from Defects or Blemishes Visible before the Candle**—A yolk that, when viewed before the candle, shows no spots or areas on its surface indicating the presence of germ development or other defects or blemishes.
21. **Practically Free from Defects and Blemishes Visible before the Candle**—A yolk that, when viewed before the candle, may show very slight blemishes within the yolk shadow.
22. **Definite but not Serious Defects Visible before the Candle**—A yolk that may show definite spots or areas on its surface, when viewed before the candle, indicative of defects but with no definite indication of germ development or other pronounced or serious defects or blemishes.
23. **Other Serious Defects**—A yolk that shows well-developed spots or areas of a character that constitute serious defects or blemishes but do not render the egg inedible.
24. **Clearly Visible Germ Development**—A development of the germ spot on the yolk of a fertile egg that has progressed to a point where it is plainly visible before the candle, as a rather definite, deeper colored circular area, or as a distinct spot on the yolk, with no blood in evidence.
25. **Blood Due to Germ Development**—Blood caused by development of the germ in a fertile egg to the point where it is visible before the candle. Such eggs are classified as inedible.
26. **Blood Clots (Blood not Due to Germ Development)**—Spots or clots of blood usually on the surface of the yolk but sometimes floating in the white. If they are small (not over $^1/_8$ inch in diameter), the eggs may be classed as U. S. Grade C. If larger and/or showing a diffusion of blood in the white surrounding them, the eggs should be classified as inedible.

TERMS DESCRIPTIVE OF THE WHITE

27. **Clear**—A clear white is one that is free from discoloration or from any foreign bodies floating in it. Prominent chalazae should not be confused with foreign bodies, such as meat spots or blood clots.
28. **Firm**—A firm white is one that is sufficiently thick or viscous to permit but limited movement of the yolk from the center of the egg when it is twirled. A firm white should have a consistency not lower than the white shown in No. 2 of the Van Wagenen Chart of Broken-out Eggs.
29. **Reasonably Firm**—A reasonably firm white is one that has a reasonably good viscous condition but not as strong a condition as a firm white. A reasonably firm white permits the yolk to move somewhat more freely from its normal position in the center of the egg and thus to approach the shell more closely when the egg is twirled. A reasonably firm white may, therefore, cause the outline of the yolk to be fairly well defined. A reasonably firm white should have a consistency not lower than the white shown in No. 3 of the Van Wagenen Chart of Broken-out Eggs.
30. **Slightly Weak**—A slightly weak white is one that has lost its firm or even its reasonably firm condition, has become less viscous and is not as clearly differentiated from the thin white when the egg is broken out. It should have a consistency not lower than the white shown in No. 4 of the Van Wagenen Chart of Broken-out Eggs.
31. **Weak and Watery**—A weak and watery white is one that is thin and generally lacking in viscosity and that, therefore, permits the yolk to move freely from its normal position in the center of the egg and to approach the shell closely when the egg is twirled. It may have a consistency lower than the white shown in No. 4 of the Van Wagenen Chart of Broken-out Eggs.
32. **Small Blood Clots or Small Meat Spots**—Spots or clots of blood usually on the surface of the yolk but sometimes floating in the white. These blood clots may have lost their characteristic red color and appear as small spots of foreign material commonly referred to as meat spots. Such blood clots or meat spots are incorporated in the egg during its formation as, or after, the yolk leaves the ovary. Such blood spots are not due to germ development.
33. **Bloody White**—An egg, the white of which has blood diffused more or less generally through it Such a condition may be present in new-laid eggs. Eggs with bloody whites are classed as inedible.

GENERAL TERMS

34. **No Grade**—Eggs of possible edible quality that fail to meet the requirements of an Official or Tentative U. S. Grade or that have been contaminated by smoke, chemicals, or other foreign material that has seriously affected the character, appearance, or flavor of the eggs are classed as "No Grade."
35. **Loss**—Eggs that are inedible, smashed, contaminated or containing bloody whites, large blood spots or large or unsightly meat spots, or other extraneous or foreign material are classed as "Loss."
36. **Inedible Eggs**—Under the Federal Food, Drug and Cosmetic Act eggs that are filthy, putrid, or decomposed, or otherwise unfit for food in whole or in part are adulterated. Eggs of the following descriptions are classed as inedible: black rots, white rots, mixed rots (addled eggs), sour eggs, eggs with green whites, eggs with stuck yolks, moldy eggs, musty eggs, eggs showing blood rings, eggs containing embryo chicks (at or beyond the blood ring stage) and any other eggs that are filthy, decomposed or putrid.

5. Processed Shells

Many years ago covering an egg with lard or other bland fat was often practiced on the farm as a method of preserving quality. Other methods, such as immersion in solutions of water-glass, were found to be far better, hence greasing has been discarded. The old-time practice, however, led to a study of the ability of mineral oil to enhance keeping quality and very definite instructions have been drawn up for its use as well as general specifications for the character of oil best adapted to the purpose.

In the early days of "sterilizing," as the process was erroneously called, heavy oils were used which had to be heated to render them sufficiently fluid to flow over the shell in a very thin film. Even so, when the egg cooled, the shell was shiny and frequently was oily to the touch. The surface of the shell was not rendered bacteria- nor mold-free. On the other hand, mold spots within the shell so frequently destroyed the edibility of the egg that the process fell into disrepute. A substitution of light mineral oil and atmospheric temperatures so reduced mold penetration and, at the same time, so effectively prevented evaporation that "processed eggs," as oiled shells are now known, are a recognized asset in commercial preservation of eggs in the shell. Oiling of very fresh eggs is of limited service in holding quality under atmospheric temperatures, but is far more efficient in reducing shrink and maintaining quality when the temperatures are from 29 to 31° F.

A typical, light, colorless, odorless mineral oil has the physical characteristics shown below:

Specific gravity at 60° F.	0.830–0.840
Saybolt viscosity at 100° F.	50–55
Saybolt color	30 plus
Pour point—max., ° F.	30
Flash-min., ° F.	285
U. S. P. acid test	Passes

"Processing" the egg shell must be done with care and accuracy. The oil is commonly heated to 100° F. to ensure the expansion of the air in the air cell, though temperatures as low as 70° F. are used successfully. The immersion in oil is for a matter of seconds only, the egg is drained for 1 or 2 min. and then placed in its filler in a regular egg case. The filled case may be put into the cooler without delay. Within 24 hrs. the shine has largely disappeared and the shell is smooth but not oily to the touch.

The oil must be kept clean. Filter out pieces of dirt or feathers by pouring it through cheesecloth. If a sludge forms, or the oil becomes brownish, filter through activated carbon. But when a distinct odor appears, as it will in time, the better plan is to discard it and use fresh oil.

6. Frozen Egg

This very important egg product is made by removing the egg meat from the shell, thoroughly mixing white and yolk, if whole egg is desired, and freezing as rapidly as possible in a suitable container, generally tin. Whites and yolks may be frozen separately.

Although freezing eggs for food as an industry began only in the late 1800's, its growth has been phenomenal. The pack in 1941 was over 237,000,000 lbs. Part of this frozen egg, however, was thawed and dried to supply the increased war demand for dried egg.

In the early years of the industry frozen egg was too often made from eggs of poor quality broken out and handled in insanitary fashion. Fortunately, that time is past and the freezing of eggs is now a respected industry under bacteriological and chemical control. Practically all large bakeries depend upon frozen egg the year around since the bulk of it is packed in the early spring when eggs are at their best and because its composition, especially the total solids and the amount of fat, is constant—an important item in the successful use of baking formulas.

Economically frozen egg is sound. It must not be forgotten that more than half of the lay for the entire year is produced from March to June, inclusive. Storing surplus eggs in the shell at temperatures between 29–31° F. has been brought to a high degree of efficiency, yet slowly deterioration does go on—while, when hard frozen, egg can be held almost indefinitely without destructive change. Also, the saving in storage space and transportation costs is very considerable.

The removal of egg meat from the shell, its proper mixing to obtain uniformity, its packaging and freezing are a specialized operation for which specialized facilities are required.

As soon as eggs for breaking are received, they should be placed in a cooler having a temperature of 32° F. The cases should be set on floor strips of 2 in. × 4 in. lumber and 1 in. of air space should be allowed *on all sides of each case.* This means that 1-in. strips of wood should be placed between cases as they are stacked. If eggs come to the packing house with a temperature of 80° F. and over, as they frequently do in summer, from 2 to 3 days under above conditions will be required to reduce them to 32° F. If the air is circulated by a fan, the cooling time is reduced by about one-half. If the room is maintained at 32° F., however, the temperature of the eggs will be down to 40° F., or thereabout, in 24 to 36 hrs., and that is a good temperature at which to candle them and send them to the breaking room. If the breaking room is as it should be, at 55–60° F., eggs so cooled, if handled expeditiously, will not sweat. Sweating is also reduced if, in the air conditioning of the breaking room, due attention is paid to keeping the air dry—just the opposite of what we desire in the egg cooler.

(*a*) *Breaking Facilities and Holding Freezer*

Egg breaking should be done in well-lighted rooms of strictly sanitary construction, well ventilated and air conditioned. The desirable temperature is between 55–60° F. The apparatus required includes a metal stand, mounting a knife blade on which the egg is cracked, and a suitable tray to support the cups to receive the egg meat. These cups are small—capacity not over three eggs—and are three in number when whites and yolks are to be prepared, and two when whole egg is being made. The breaker cracks and breaks three eggs into each cup, then lifts both, smells each one and, if found satisfactory, dumps the contents into a collecting pail or can and returns the cups to the breaking tray to be refilled.

If, either by appearance or odor the contents of a cup are unsatisfactory, the whole tray is sent to the washroom to be cleaned and sterilized, and the breaker washes and dries her hands before returning to her place In case of a doubtful egg, the cup containing it is sent to the "referee"—an experienced breaker with a sharp sense of smell who decides upon its edibility.

Fig 49 —Breaking eggs for the frozen or dried product

All apparatus coming in contact with inedible liquid egg must be sterilized before it is again used. Also, at least twice a day, preferably at noon and again at the end of the day all the breaking room apparatus, including collecting pails for liquid egg and carriers for shell eggs, should be washed and steamed. Of course, breaking trays and their adjuncts must be sterilized.

Removing egg meat, especially if it has dried, from apparatus is much more difficult than removing milk or cream. Therefore the greatest care should be taken to have all surfaces smooth and free from sharp angles. The washroom should be as carefully planned as the breaking room. It should be well lighted and a suction fan should remove the steam which, if in sufficient amount to cleanse properly, will be too much for the workers in the premises. Also, plenty of space should be allowed. The cramped, dark, badly ventilated washrooms of some egg-breaking plants are fertile sources of high bacterial counts in the product.

The layout of an egg-breaking plant should ensure economy and ease of operation, compactness, and top-notch sanitation. Since the breaking room and candling room should be maintained at temperatures between 55–60° F., their walls must be insulated. While the floor plan must conform to the individual building, certain relations should be maintained. A generally useful plan is given in Fig. 50. A list of equipment to guide egg breakers is given in this bulletin and is quoted below.

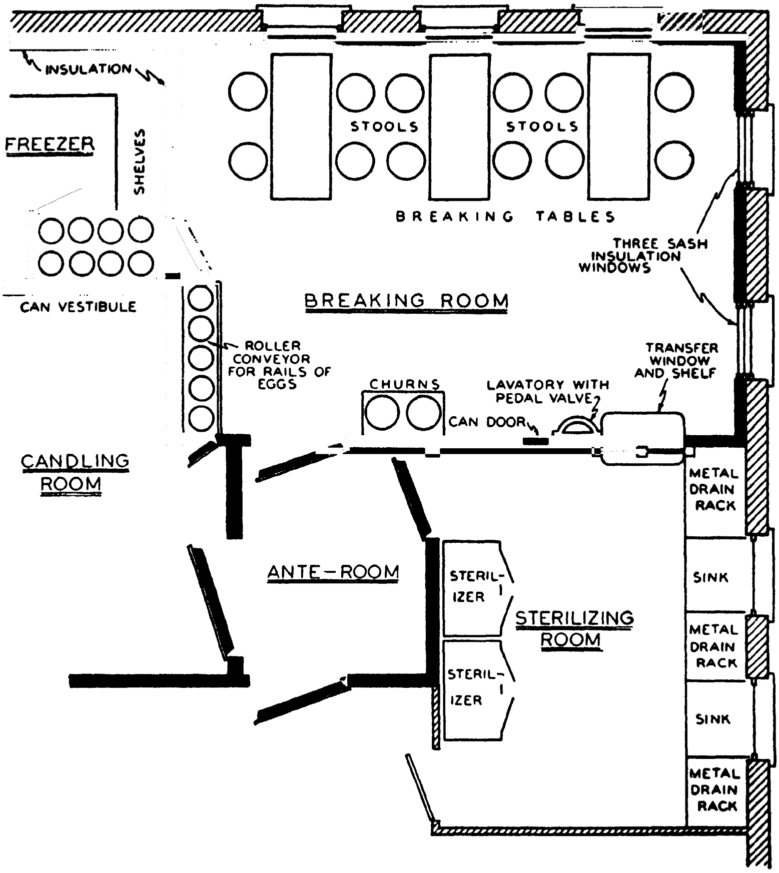


Fig. 50.—A typical layout of breaking and sterilizing rooms.

If whole egg is desired, the more or less mixed whites and yolks are put into a suitable mixer, or "churn" as it is usually called, and made into a homogeneous, thick, creamy mass. Before or after churning the egg is passed through a fine wire screen to remove the strings of chalazae and fragments of shells. If yolks or whites are desired, the separated eggs are handled in their respective churns. Shells and chalazae are removed as above.

From the churn the products are delivered directly to containers and transferred at once to a sharp freezer.

"The egg must be *frozen hard* as *quickly as possible*. Many a can of fine egg has been wrecked because of such a slow freeze that the core was sour before it congealed.

"Since the liquid next to the sides of the can freezes first, and the freezing is toward the center, there is of course a migration of the solids toward the center. That means a lower and lower freezing temperature.

OUTFIT LIST FOR EGG-BREAKING PLANTS[a]

Item	Sold or made by	Number required
Equipment Purchasable on the Market:		
Breaking room:		
Pails for eggs	Hardware firms	3 to 6 per breaker
Pails for liquid product	Hardware firms	$1^1/_2$ per breaker
Leaker trays	Special tinning firms	3 per candler
Separators	Special manufacturers	2 per breaker
Glass cups	House-furnishing stores	12 per breaker
Storage cans for shells	House-furnishing stores	
Holders for toilet paper	House-furnishing stores	1 per breaker
Rack for paper towels	House-furnishing stores	1 per room
Spoons	House-furnishing stores	$1^1/_2$ per breaker
Stools		
In wood	House-furnishing stores	1 per breaker
In iron	Hospital supply firms	
Lavatory with pedal-valve attachment	Plumbing supply firms	1 per room
Scales	Scale manufacturers	1 per churn and 1 for whites
Cans	Can manufacturers	According to output
Refrigerated churn	Dairy supply firms	
Dasher for hand churning	Dairy supply firms	
Uniform for men	Dairy supply firms	3 per worker
Waterproof apron	Dairy supply firms	1 for each operator in washroom
Sterilizing room:		
Sinks	Dairy supply firms	
Sterilizers	Dairy supply firms	
Cup rinser	Dairy supply firms	
Can rinser	Dairy supply firms	
Cup-washing machine	Dairy supply firms	
Equipment Made to Order:		
Breaking room:		
False bottoms for pails for shell eggs	Tinner	1 for each pail for shell eggs
Breaking trays	Tinner and machinist	$1^1/_2$ per breaker
Breaking knives	Machinist	6 per breaker
Uniforms for women	Seamstress	3 per worker
Breaking table	Tinner	1 unit table per 2 breakers
Funnels	Tinner	1 per unit table
Shell cans	Tinner	1 per unit table; few additional
Shell tampers:		
Small	Tinner	1 per breaker
Large	Tinner	1 to 2 per room
Cup trays	Tinner	1 per 24 cups
Knife racks	Machinist or tinner	1 per 36 knives
Churn without refrigeration	Tinner	
Sterilizing room:		
Draining racks for sink	Tinner	

[a] M. K. Jenkins, U. S. Dept. Agr., Bur. Chem., *Bull.* 663 (May, 1922).

"When low temperatures were not as readily available as now, it was the custom to fill the can about two-thirds full and freeze. Then the remaining third was filled and the freezing completed. By this means a flat surface was obtained; whereas, when the filled can was frozen, the expansion of the central core made a humped surface. This practice, now that we have temperatures as low as −20° F. commercially available, has been discontinued.

"If a temperature of 0° F. or a little below is maintained, 30 lbs. of canned egg in a freezer having natural air circulation may be expected to become solidly frozen in about 72 hrs. Since desiccation of the product cannot take place, however, it is permissible to air blast the cans, in which case, only half the time is required. Or, the shelf type of freezer, such as was developed years ago for the better freezing of fish, can be used to freeze liquid egg. In this case the egg on the bottom of the can freezes most rapidly.

"The liquid egg should be as cold as possible when it reaches the freezer. To that end some churns are jacketed with cold, circulating brine. But the time in the churn is very short. The continued refrigeration from receipt in the cooler to the final freezing is the thing that counts.

"The practice of initially freezing under conditions especially adapted to the work to be done and then transferring to another freezer for holding is growing. Since an air blast can be used for initially freezing when the package is a metal can it is more economical to freeze in a tunnel and then transfer to a holding room at 0° F. than to maintain the much lower temperature required by still air for the initial freeze.

"The accepted temperature for holding is 0 to −5° F."[36]

Until the war emergency reduced the supply of tin and steel plate for civilian purposes, frozen eggs were packaged in 30-lb. tin cans. When the metals shortage came, the industry must needs turn to paper products. Substitutes for tin cans have been produced—not as satisfactory as tin, but usable under the circumstances. These packages are of water-resistant paper products, some cylindrical, some rectangular, with liners which are watertight. Generally when shipped the container is protected by a fiber carton—either solid fiber or corrugated board. Freezing is somewhat slower than in metal.

(*b*) *The Product*

When defrosted, frozen whole egg is a smooth, thick, yellow cream which has good lifting power and can be used by bakers and confectioners for all general purposes. As commercially produced, "whole" egg seldom contains white and yolk in "natural" proportions. Also, in the early spring when evaporation of the water through the shell is slow, eggs coming to the breaking room will contain more water than later in the summer when high temperatures will have accelerated evaporation. Since one reason for the use of commercially prepared frozen eggs is to obtain constant composition, specifications now generally require a minimum of 25.5% of egg solids.

The A. O. A. C. method for the determination of solids in egg is too time consuming to serve as a means of regulating the amount of egg solids in whole egg. There must be a method so quickly used that additional white or yolk, as the case may be, can be added to the egg mixture to bring it to standard *while it is in the churn.* The method used is based on the refractometer. Total solids in per cent are read directly on the scale of a special instrument which also has a temperature control. The determinations are accurate to 0.5% or less and so simply made that one neat, careful girl can easily test each churn and keep up with fifty breakers.[37]

---

[36] M. E. Pennington, *Refrig. Eng.*, **42,** 6 (1941).

Egg white must be opalescent and of good, clear color and free from yolk and from pieces of shell. It is strained to remove pieces of chalazae.

The yolk of egg, removed from the shell, mixed thoroughly and frozen, will thaw to a thick, smooth, golden paste. It thickens if held hard frozen for more than a few weeks and so becomes more difficult for the baker to incorporate into his mixtures. The introduction of sugar, salt, glycerol, or some such compound will prevent this gelation and give a more easily flowing product. Up to 10% of salt or of sugar may be added to egg yolk. Salted yolk is generally used by mayonnaise makers and sugared yolk by cake bakers and confectioners. The amount of egg fat which the product must carry depends upon the use to which it is to be put. Mayonnaise, for example, generally requires a richer yolk than does the usual cake. Ordinarily, however, the solids in frozen egg yolk must be at least 43%.

7. Dried Egg

Before the first World War the United States had an egg-drying industry—not as extensive as its frozen-egg industry but enough to supply most of this country's needs. After that war, however, the industry, because of increased cost of labor and raw materials and for other reasons, was transferred to China and Shanghai became the chief source of this product. Much of the egg was dried on moving belts in heated, dried air—some on pans. Shortly before the transfer of the industry to China, egg was dried by spraying it as a mist into a heated chamber where if fell as a fine yellow snow. This method was rapidly gaining ground when dried egg "went to China."

In World War II, because of overseas demands and the lack of shipping and of refrigeration, dried egg came again to the fore. An enormous industry sprang into being almost overnight. During 1941 the output was 45,280,000 lbs. During 1942, the tonnage grew to 235,649,000 lbs. In the spring of 1941 there were 16 egg-drying plants in the United States.[38] In November, 1941, there were about 60. At the close of 1942 the number had increased to about 130.

It is obvious that with such phenomenal expansion and under war conditions the industry has solved most of its difficulties by the cut-and-try method, rather than by approved scientific procedures. Many of the new plants knew nothing of egg breaking, a task requiring specialized technique and exemplary sanitation. Engineers were called upon to build drying machinery to handle tons of egg without any preliminary information except such as was gained in the dehydration of milk. During the next few years there should be a voluminous literature on the drying of eggs, but at the present time there is very little.

As in the dehydration of other animal and plant tissues the extremely rapid evaporation of water so reduces the temperature of the substance that the temperature of the air can be much higher than that of the material being dried. In other words, there is a wide difference between the temperature of the dry and the wet bulbs. The temperature of the wet bulb is approximately the temperature of the material. Of course there must be a proper flow of dry air ready to absorb and remove the moisture driven out of the egg, and there must be a continuous supply of dry, heated air sufficient to do the work. In spray drying some form of atomizer must deliver the liquids to the dehydrating chamber in such a finely divided state that its dehydration is almost instantaneous. The fine, dry powder of the desired water content is then collected, cooled, and packaged without further processing.

---

[37] M. I. Bailey, *Ind. Eng. Chem., Anal. Ed.*, **7**, 385 (1935).

[38] N. Koenig, *U. S. Egg Poultry Mag.*, **48**, No. 1, 39 (1942).

The idea that low-quality egg can be dried to high-quality product is fallacious. Egg gains nothing by drying. This fact had to be demonstrated to certain processors and too much stress cannot, even yet, be laid upon it. Also, it must be handled, from shell to package, under strict bacterial cleanliness and unbroken refrigeration. True, the process of dehydration kills many bacteria. Others die during holding, if the moisture content is kept low. But some organisms do survive and, what is more important, a dried egg which has supported a heavy growth of bacteria or mold will have undergone changes which are detrimental to quality before drying and which are not camouflaged by dehydration. Therefore, the processor should make counts of viable bacteria in the liquid egg going to the drier as well as in the dried product. Also, the count in the dried egg should be made promptly—probably within 3 days after drying and cooling. The flora is varied, of course. Fecal organisms do survive drying, as do air and water and soil saprophytes. To give all these groups a chance to develop, 32° C. has been selected by Stuart, Hall, and Dicks[39] as the incubation temperature and 72 hrs. as incubation period. One-gram samples were weighed into 99 ml. of sterile physiological salt solution as the first dilution, and Bacto-Tryptone glucose agar as given in the American Public Health Association's "Standard Methods for the Examination of Dairy Products, 1941," for dried eggs was used for plating. Special types of bacteria, such as coliform and lipolytic organisms, were tested according to accepted methods. The total count varies from a few thousand organisms to hundreds of millions, depending upon the microbial population of the original raw material, the efficiency of the sanitation of breaking, mixing, and conveying to the drier and the type of dehydrator. While a high count denotes poor quality, a low count may be due to overheating, which results in lowered solubility and palatability. All other things considered, a count of 20,000 to the gram or less is indicative of very clean egg. Probably 150,000 to the gram is good quality, though there is considerable hesitation on the part of food technologists to fix rigid bacterial limits.

Stuart *et al.*, in the course of a study on storage changes in dried egg have determined moisture according to A. O. A. C. methods, free fat acids, combined amino and amide nitrogen, total free carboxyl groups, solubility, and *p*H. The authors state that *p*H decreased in storage as the concentration of the suspension is lowered. For example, freshly dried egg in a suspension of 1 in 4 gave *p*H 8.12, and after 6 months' storage the *p*H had fallen to 7.00. However, in suspension 1 to 100 fresh powder gave 8.56 and after 6 months' storage the figure had dropped to 7.64. The moisture content rose (at 30° C., relative humidity 70%) in 60 days from 3.80 to 5.29%; the total nitrogen, the total soluble nitrogen, and the crude albumen nitrogen fell markedly. The higher the humidity, the greater the loss. Solubility of the stored powder decreased as the percentage of moisture increased.

---

[39] L. S. Stuart, H. H. Hall, and E. E. Dicks, *U. S. Egg Poultry Mag.*, **48**, No. 12, 629 (1942).

All these changes were accentuated when the original sample of dried egg powder contained more than 5% of moisture. The authors' conclusions regarding the bacterial count are "Changes in the total bacterial count, as measured by the dilution plate method, are influenced by the amount of moisture initially present in the dried egg powder. When the moisture concentration is 5.0% or less, bacterial death appears to be greater as the moisture content is reduced. With moisture concentrations greater than 5.0%, the reverse seems to be true, that is, until the moisture concentration becomes high enough to allow for bacterial growth. Death of bacteria in powdered dried egg at moisture concentration greater than 5.0% appears to be associated with the chemical changes involved in the loss of solubility."

A later study of storage changes in dried eggs[39a] states that removing the glucose from egg white prior to dehydrating gives a permanently stable product, but does not materially affect the stability of the yolk. Low moisture levels—0.5 to 1%—improve keeping quality in dried albumen and whole egg, but not in dried yolk. Adjusting the *p*H of whole egg to 6.5 prior to drying and then reducing the moisture to less than 1% gives a product with decidedly superior keeping qualities. In dried eggs, as in other dehydrated food products, the trend is toward less and less moisture.

Storage

Even when the moisture content is 5% or less, atmospheric temperatures of 50° F. and below are recommended if the palatability and solubility of the egg powder are to be preserved. Fifty degrees is the maximum temperature to which egg powder should be submitted for any length of time. Putting egg powder fresh from the drier with a possible temperature of 110° F. into the large barrels (190-lb. capacity) and allowing cooling to proceed naturally is detrimental to quality. Therefore, forward-looking driers of egg are developing methods of quick cooling of the powder so that it enters the barrels below 70° F. and is then placed at once in a refrigerated cooler at approximately 32° F. where it is held until it goes into consumption.

A method for the determination of palatability has been developed by Wilson and Slosberg[40] with a jury of four tasters. These gentlemen reconstitute 12 g. of dried egg with 50 ml. of distilled water, mix on a mechanical shaker, and cook in a household egg poacher for 5 to 6 min. The water should boil gently. When the egg is suitably "scrambled" it is transferred to plates and tasted. The rating is from 1 to 10, as used by the British. A score sheet follows:

[39a] G. F. Stewart, L. R. Best, and B. Lowe, *Proc. Inst. Food Technologists*, St. Louis, Mo., **June, 1943**, pp. 77–89.

[40] R. V. Wilson and H. M. Slosberg, *U. S. Egg Poultry Mag.*, **49**, No. 1, 21 (1943).

FLAVOR SCORE

DRIED WHOLE EGG

Score			
10	Excellent	Comparable to fresh egg	Suitable for household use
9	Very good		
8	Good		
7	Slight storage flavor		
6	Storage flavor		
5	Slight "off" flavor		Not acceptable for household use
4	Definite "off" flavor		
3	Old and unpleasant flavor		
2	Very old and unpleasant flavor		
1	Inedible		

Since palatability is a prime asset in egg of any sort and especially in dried egg where it must be heavily leaned upon in determining quality, Pearce and Thistle[41] have devised a method wherein fluorescence is a measure of palatability. The egg is defatted by shaking with petroleum ether, chloroform, or 3:1 petroleum ether-absolute alcohol. The defatted powder is extracted with 10% potassium chloride solution and the fluorescence of the filtrate determined with a Coleman photofluorometer using the standard B_1 vitamin filter which transmits light in the region of 365 $m\mu$. The instrument was standardized to a reading of 50.0 for the fluorescence of a solution containing 0.200 γ of quinine sulfate per ml.

These readings were assessed by a panel of 14 people who tasted each sample. Statistical analyses of panel taste scores indicated that 14 different people are necessary to give a sound rating, though 7 people give a fairly good judgment. An organoleptic rating which gave 3.77 showed a fluorescence of 55.7, while a rating of 8.38 points showed 15.0. It is needless to comment upon the desirability of a reliable, practical objective method by which the palatability of dried egg powder can be assessed.

8. Transportation of Eggs

Some of the fundamental requirements for successful transportation of eggs in the shell are: (1) new fillers, flats and cases; (2) six cement-coated 3-penny nails at each corner of the sides and bottom of the standard case; (3) tight stowing of cases in the car; (4) self-bracing of incomplete layers rather than braces nailed to the walls of the car; (5) last, but not least, eggs with sound shells which fit the cells of the filler. All of these and other details of the egg package and carloading and hauling are discussed in United States Department of Agriculture Bulletin, "Prevention of Breakage of Eggs in Transit."[42] At the time this study was made from 1 to 2% of eggs were damaged in transit. Later (1926) another study was made under the auspices of the United States Department of Agriculture,[43] according to which the average damage per case "amounted to 1.37 new leakers, 2.22 new checks, 1.35 new blind checks, 0.41

---

[41] J. A. Pearce and M. W. Thistle, *Can. J. Research*, **20** (Sept., 1942).

[42] M. E. Pennington, H. A. McAleer, and A. D. Greenlee, U. S. Dept. Agr., *Bull.* **664** (April, 1918).

[43] R. Slocum, U. S. Dept. Agr., *Circ.* **391** (Aug., 1926).

blind checks changed to checks, 0.04 blind checks changed to leakers, and 0.1 checks changed to leakers." The money loss, in terms of the value of eggs at the time the study was made, was less than 2 cents per case. Egg cases made from fiberboard, especially those for holding 15 doz. eggs, have been in commercial use for a number of years. The demand for wood during World War II has, however, brought the 30-dozen fiber egg case into general use and the number of such cases is constantly increasing. The inside dimensions are the same as those of the standard wood case; the outside dimensions are usually a little smaller.

Eggs should be, and generally are, shipped in refrigerator cars. The shocks of the haul are minimized in these cars of heavier construction. To ensure careful treatment during switching and in terminal yards, however, each car carries a placard with the word "Eggs" in large.letters.

Refrigeration during transportation, as in every other item of marketing eggs, is essential if quality is to be maintained. Usually the temperature in the car will be between 45–50° F., assuming the bunkers are well iced.

In midsummer many shippers add from 1 to 2% salt to the ice when initially icing to cool more quickly the car and the eggs. Even when the eggs have been in a cooler for several days the light salting is advantageous. When warm eggs are loaded into an iced car in summer, about 100 hrs. are commonly required to reduce the eggs in the cases to 45° F.

During the winter season the insulated walls of the refrigerator car are a protection against freezing. Supplemental insulation in the form of straw under and around the load is used in very cold weather and heaters, provided by the car line, are an additional means of guarding against freezing in transit.

Chapter XVI

EDIBLE OILS AND FATS

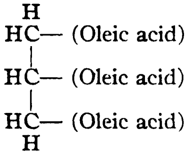
ALTON E. BAILEY

New Orleans, Louisiana

I. THE NATURE OF OILS AND FATS

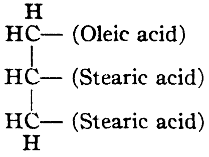
1. The Fatty Acids and Their Glycerides

Oils and fats consist essentially of glycerol esters of the fatty acids, or triglycerides. From the structural standpoint, each triglyceride molecule may be considered to be formed by the condensation of one molecule of glycerol with three molecules of fatty acids. The three fatty acids may be identical, in which case the product is a simple triglyceride, or they may be different, in which case it is a mixed triglyceride. Natural triglycerides are usually mixed; simple triglycerides apparently occur in appreciable quantities in only a limited number of oils.



$$\begin{array}{l} \;\;\text{H} \\ \text{HC— (Oleic acid)} \\ \;\;\,| \\ \text{HC— (Oleic acid)} \\ \;\;\,| \\ \text{HC— (Oleic acid)} \\ \;\;\text{H} \end{array}$$

Simple triglyceride
(triolein)



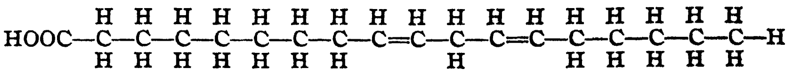
$$\begin{array}{l} \;\;\text{H} \\ \text{HC— (Oleic acid)} \\ \;\;\,| \\ \text{HC— (Stearic acid)} \\ \;\;\,| \\ \text{HC— (Stearic acid)} \\ \;\;\text{H} \end{array}$$

Mixed triglyceride
(α-oleodistearin)

The molecular weight of the alkyl residue of the glycerol portion of a triglyceride molecule (C_3H_5) is 41. The combined molecular weight of the fatty acid radicals ($R \cdot COO$) comprising the remainder of the molecule is usually not less than about 800. The fatty acids thus comprise approximately 95% of the total molecular weight of the glycerides; consequently, their influence on the character of the glycerides is very great, and the chemistry of fats and oils is to a considerable extent the chemistry of their component fatty acids.

The naturally occurring fatty acids generally consist of normal, monobasic, aliphatic compounds, consisting of a single carboxyl group attached to a straight carbon chain. The various fatty acids differ from one another in the number of carbon atoms in their chains and in the number of ethylenic linkages, or double bonds, between the carbon atoms. The position of the double bonds also affects the properties of the acids. The geometrical configuration, for example, *cis-trans*-isomerism, likewise affects the properties of fatty acids, although in the acids generally found in natural edible oils isomerism of any type is of little importance.

All naturally occurring fatty acids have an even number of carbon atoms. The C_{16}- and C_{18}-acids predominate, but some oils have acids with as few as four carbon atoms and others have as many as 26 or more. When double bonds occur there are usually one or two in number, but may be as many as five or six.



$$\mathrm{HOOC}-\underset{H}{\overset{H}{C}}-\underset{H}{\overset{H}{C}}-\underset{H}{\overset{H}{C}}-\underset{H}{\overset{H}{C}}-\underset{H}{\overset{H}{C}}-\underset{H}{\overset{H}{C}}-\underset{H}{\overset{H}{C}}-\overset{H}{C}=\overset{H}{C}-\underset{H}{\overset{H}{C}}-\overset{H}{C}=\overset{H}{C}-\underset{H}{\overset{H}{C}}-\underset{H}{\overset{H}{C}}-\underset{H}{\overset{H}{C}}-\underset{H}{\overset{H}{C}}-\underset{H}{\overset{H}{C}}-H$$

C_{18}-Acid with two double bonds (9:10, 12:13 linoleic acid)

TABLE 101

OCCURRENCE OF THE MORE IMPORTANT FATTY ACIDS IN COMMON EDIBLE FATS AND OILS

Fatty acid	Number of carbon atoms	Number of double bonds	Occurrence (in terms of % by weight)		
			0–5	5–25	Over 25
Butyric	4	0	Butter	None	None
Caproic	6	0	Butter	None	None
Caprylic	8	0	Butter	Coconut, palm-kernel	None
Capric	10	0	Butter	Coconut, palm-kernel	None
Lauric	12	0	Butter	None	Coconut, palm-kernel
Myristic	14	0	Many oils and fats	Butter, coconut, palm-kernel	None
Palmitic	16	0	Rapeseed	Many oils and fats	Palm, lard, tallow
Stearic	18	0	Many oils and fats	Lard, tallow	Cocoa butter
Arachidic	20	0	Many oils and fats	None	None
Behenic	22	0	Peanut, rapeseed	None	None
Lignoceric	24	0	Peanut, rapeseed	None	None
Palmitoleic	16	1	Probably many oils	Fish, whale	None
Oleic	18	1	None	Coconut, palm-kernel, fish	Most oils and fats
Gadoleic	20	1	Probably many oils	Fish	None
Erucic	22	1	None	None	Rapeseed
Linoleic	18	2	Coconut, palm-kernel, cocoa butter	Olive, rapeseed, palm, lard, fish, whale	Peanut, cotton, sunflower, corn, soybean
Linolenic	18	3	Soybean, rapeseed	None	None
Arachidonic, clupanodonic, etc.	20–24	4–6	Lard	Whale	Fish

Because of the fact that the double bonds provide points in the molecule for the addition of oxygen, hydrogen, iodine, or other reactive substances, the acids with double bonds are termed unsaturated. Those acids which do not contain double bonds are referred to as saturated. The degree of unsaturation of an oil depends upon the average number of double bonds in its fatty acids.

The introduction of double bonds into a fatty acid results in a lowering of the melting point, so that the melting point of a fat or oil is in general an indication of its degree of unsaturation. The melting point depends to a lesser degree upon the length of the carbon chain, increasing as the chain lengthens.

A considerable number of different fatty acids are known, but the number found in significant quantities in edible oils and fats is not large, and many important oils contain primarily only the four most common acids, namely, palmitic, stearic, oleic, and linoleic (see Table 101). From a practical standpoint the occurrence of fatty acids in minor amounts is not usually important, since the properties of a minor acid will not in general be sufficiently different from those of the major acids to seriously influence the over-all characteristics of the oil. If the minor acid is more unsaturated than the other fatty acids, however, it may markedly influence the stability of the oil. Ability to resist atmospheric oxidation is highly important in edible oils, and the susceptibility of fatty acids to oxidation increases greatly as their degree of unsaturation increases.

2. Nonglyceride Components

The oils and fats of commerce contain small amounts of various substances other than glycerol esters, some of which, even in traces, considerably affect the character of the product. Certain of these substances have marked antioxygenic properties, and their presence in vegetable oils appears to serve the specific purpose of protecting the oil from deterioration during the normal life of the parent seed or fruit. Because of their greater content of antioxygenic substances, vegetable oils are generally more stable than animal oils of a corresponding degree of unsaturation.

Pure triglycerides are colorless, odorless, and tasteless, but all natural oils and fats are colored to some extent by the presence of oil-soluble pigments. The natural oils and fats also have distinctive odors and flavors resulting from the presence of traces of characteristic impurities. Some oils, as, for example, butterfat, olive oil, and lard are prized for their characteristic flavors and odors. In other oils, including cottonseed, soybean, peanut, sunflowerseed, palm, coconut, palm-kernel, whale, fish, etc., the natural flavors and odors are less prized and may even be objectionable. In Occidental countries these oils are generally processed to remove the natural odor and flavor before they are consumed.

All oil- and fat-bearing tissues, both animal and vegetable, contain lipolytic or fat-splitting enzymes, which in the presence of moisture have the power of hydrolyzing the oil in nonliving tissue, to produce in it both glycerol and fatty acids in their free form. Because of the impossibility of expressing the oil before at least some slight splitting has taken place, all fats and oils, as commercially produced, contain a certain amount of free fatty acids. The amount may vary from as little as 0.10% or less, as in the case of lard or tallow rendered from freshly killed animals, to 10% or more in palm oil, fish oils, or other oils or fats which have been subjected to prolonged enzyme action.

3. Distinction between Fats and Oils

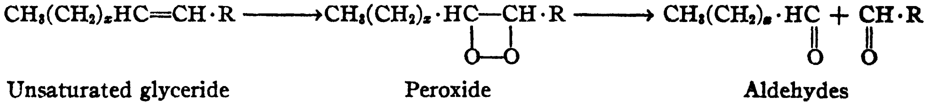
No sharp differentiation can be made between fats and oils, since the difference between the two is purely a matter of environment. Oils are ordinarily liquid and fats are ordinarily solid, but at the proper temperatures all oils will solidify and all fats will melt. As a matter of fact, the designation of fats as solids is somewhat misleading; at ordinary temperatures fats are actually plastic solids, and consist of a liquid in intimate mixture with many very small solid particles. At 70° F., for instance, only about one-third of the glycerides of lard are in a solid state. The terms oil and fat may consequently be used interchangeably for all purposes of discussion except where the fluidity or plasticity of the product is in question.

4. Spoilage of Fats

Deterioration and spoilage in oils and fats is essentially different from that occurring in most other food products. Microorganisms seldom play an important role in fat spoilage. The unpleasant flavors and odors which develop in spoiled fats are usually the result of atmospheric oxidation.

The complete mechanism by which fats become rancid is complex and not as yet fully understood, but it appears to be generally agreed that the first step is the addition of oxygen at a double bond in an unsaturated fatty acid chain, to form a peroxide. The resultant peroxides are highly reactive and likewise unstable, and readily decompose or react with one another, with the eventual formation of a variety of degradation products of relatively low molecular weight and strong flavor and odor.

The following reaction may be considered typical:



$$CH_3(CH_2)_xHC{=}CH\cdot R \longrightarrow CH_3(CH_2)_x\cdot \underset{O\text{—}O}{HC\text{—}CH}\cdot R \longrightarrow CH_3(CH_2)_x\cdot \underset{\underset{O}{\|}}{HC} + \underset{\underset{O}{\|}}{CH}\cdot R$$

The initial combination of fat with oxygen is believed to result from the operation of a chain mechanism, in which each freshly formed peroxide molecule releases the energy required for the formation of a new peroxide. The activating energy necessary for the first step of oxidation is thus transmitted from molecule to molecule, and an initial small absorption of energy will result in a relatively large amount of peroxide formation, unless the chain is broken by absorption of this energy in extraneous reactions.

The typical odor of rancidity is undoubtedly a composite of a number of different products, but the aldehydes of medium molecular weight, particularly heptylic and nonoic aldehydes, are the compounds with which rancid flavor and odor are most definitely associated. The development of rancidity is not necessarily accompanied by hydrolysis of the fat, and free acidity in a fat is no indication of the presence or absence of rancidity.

Certain oils, notably soybean, rapeseed, lard, whale, and fish, are subject to a characteristic flavor reversion, caused by a very slight degree of oxidation. Reverted flavors may in some cases be quite unpleasant, but they should not be confused with rancid flavors, as they represent a degree of oxidation far short of that required for the development of true rancidity. Flavor reversion is most noticeable in products which have been previously deodorized. It appears in hydrogenated and unhydrogenated products alike, and its course, unlike that of rancidity development, is relatively unaffected by the presence of antioxidants. It appears to be associated with the presence of unsaturated acids with more than two double bonds.

5. Antioxidants

The ease and rapidity with which an oil will oxidize depends primarily upon its content of reactive double bonds, but is also considerably influenced by the presence of certain accessory substances. These substances are termed pro-oxidants or antioxidants, according to whether they favor or inhibit oxidation. The natural occurrence of strong pro-oxidants in oils is more or less questionable, but all oils contain natural antioxidants. They are effective in extremely low concentrations, of the order of a few hundredths or even thousandths of a per cent on the basis of the oil. They are stable to heat and are generally unaffected by the commercial processing treatments of hydrogenation and deodorization.

The chain theory of fat oxidation explains the great effectiveness of antioxidants in small concentrations by supposing that the antioxidant molecules are able to absorb the activating energy of the oxidation reaction, and thus break the reaction chain.

A number of metals, including iron, are more or less strongly pro-oxidant. Copper is an extremely powerful pro-oxidant, and the use of copper or copper-bearing alloys is generally avoided in the manufacture of equipment for the processing of edible fats and oils. It is probable that pro-oxidants function mainly through their destructive effect on the antioxidants.

Because of their complex nature and their very low concentrations in the oils, antioxidants are very difficult substances to investigate, and few pure antioxygenic compounds have been isolated from natural sources. Among the few naturally occurring pounds whose antioxygenic activities appear to have been well established are cephalin and the tocopherols. The *o*- and *p*-hydroxy benzene compounds, including catechol, hydroquinone, and pyrogallol, are very effective in inhibiting oxidation when added to certain oils. It appears probable that many of the natural antioxidants are high molecular weight compounds of more or less related structure, and that their active portion consists of an aromatic nucleus with substituted hydroxyl groups. Hydrogenation of an oil often increases the effectiveness of its antioxidants, possibly through the reduction of quinones to hydroquinones. Many non-fat-soluble substances act as antioxidants when dispersed in the fats in aqueous solution. Thus ordinary sugar, for example, has a strongly stabilizing effect on the fats in sweet biscuits and similar products. The phenomenon of synergism or coaction is very common among antioxidants. The activity of two or more different antioxidants in combination is often much greater than would be expected from their individual activities. As a consequence, some fats respond much more favorably to the addition of antioxidant substances from foreign sources than do others. Oleo oil, for example, is much better stabilized by commercial lecithin than is lard, presumably because of the synergistic action of the tocopherols or carotene which are present in oleo oil.

6. The Role of Fats in Human Nutrition

Fats are the most concentrated source of energy in the diet, furnishing about 9 calories of energy for each gram consumed, as compared with the 4 calories each furnished by carbohydrates and proteins. The food consumed by a human being will normally contain from 2 to 5% of fat, and this fat will usually provide from 20 to 30% of the total caloric intake. The average per capita consumption of fats in the United States is about 60 g., or 0.13 lb., daily.

Carbohydrates are interchangeable with fats as a source of energy only to a somewhat limited degree. Severe restriction of the fat intake, such as that often imposed on war-stricken populations, leads to a very definite fat hunger. This phenomenon is probably due in part to the fact that low-fat meals are absorbed by the body in an abnormally short time, and hence are unable to effect a normal delay in the sensations of hunger. It seems evident, however, that the natural craving of the human organism for fats has other and more deep-seated origins. Fats and their component acids appear to perform a number of vital functions peculiar to themselves. Some of the unsaturated fatty acids appear to be essential building stones for vital body cells. These acids apparently cannot be synthesized by the body from carbohydrates, but must be present in the fat as it is consumed. Fats appear to perform an accessory role in a variety of metabolic processes, including the absorption of calcium, thiamine, carotene, and lactose. It is significant that four of the vitamins, A, D, E, and K, are soluble in fat rather than in water.

The relative nutritive values and digestibilities of the various commercially available oils and fats have been subjects of much lay argument and discussion and many spectacular claims, most of which are quite unjustified by the facts. In general, there is a tendency to exaggerate the differences existing among the various fats. It is true that the low molecular weight components of butterfat are somewhat more easily assimilated than are most other fats, and that low melting point oils and fats are absorbed somewhat more completely than are fats of high melting point. In the case of human beings in normal health, however, these differences cannot be said to be important. Millions of Orientals and Southern Europeans who are healthy and vigorous live on a diet practically devoid of butterfat; and even the very high melting fats of beef and mutton appear to be digested with sufficient ease by normal persons. The only really important individuality in the edible fats from a nutritional standpoint is in the appreciable content of vitamins A and D of butter.

7. Non-nutritional Functions

The unique physical properties of fats and oils enable them to perform certain important non-nutritional functions in the dietary of men. Large quantities of fats are used in the preparation of baked goods, including bread, cakes, crackers, biscuits, cookies, and pastries of various sorts. In this class of edible products, the fat not only contributes to their food value, but also provides a highly essential lubricating action. Its dispersion throughout the body of the dough prevents the gluten in the flour from compacting into a hard, refractory mass, and enables the product to assume its characteristic form and texture during the baking process. For most baked goods, a plastic fat is preferable to liquid oil, as it is relatively free from the surface tension effects that tend to cause agglomeration and coalescence of liquid droplets, and can, therefore, be maintained in a more highly dispersed form in the dough. A plastic fat also is capable of entrapping air within it during the mixing process. This air, under the influence of the heat of the oven, contributes a leavening action, which is highly important in the manufacture of certain baked products, particularly cakes.

In the frying of foods, the role of the fat is essentially that of a heat transfer medium, which serves to transmit heat from the stove or burner to the surface of the food. Fats may be safely heated to a very high temperature; consequently cooking in them is very rapid. Frying has the advantage over other methods of cooking of neither drying the food excessively nor leaching its water-soluble constituents.

One highly important function of edible oils and fats is closely associated with the phenomenon of taste. The exact mechanism of their contribution to food flavor is obscure, but it is an undeniable fact that most foods of a naturally low fat content are a great deal more pleasing to the average palate after the addition of a liberal quantity of fat or oil. The action of the oil is in this case not unlike that of a condiment. It is noteworthy, however, that an oil may be capable of greatly enhancing the flavor of other foods without having any flavor whatsoever of its own. The physiological aspects of this somewhat remarkable phenomenon do not appear to have been investigated, but the fact itself is liberally attested to by the widespread use of perfectly neutral oils, as, for example, highly deodorized cottonseed oil, for seasoning cooked vegetables, salads, sauces, gravies, etc.

II. SOURCES AND PRODUCTION OF OILS

1. Sources of Oils and Fats

As oils and fats are essential constituents of all forms of both plant and animal life, they are widely distributed in nature. The edible oils and fats which appear on the market in significant quantities, however, are derived from a relatively few sources as follows:

(*a*) *The milk of domestic animals:* Butterfat.
(*b*) *The bodies of domestic animals:* Lard, tallow (and its equivalents, oleo stock, oleostearine, and oleo oil).
(*c*) *The bodies of marine animals:* Whale oil, fish oils (sardine, menhaden, etc.).
(*d*) *The fruit coats of perennial plants:* Olive oil, palm oil.
(*e*) *The seeds of perennial plants:* Coconut oil, palm-kernel oils (including babassu, cohune, etc.), cocoa butter.

(*f*) *The seeds of annual plants:* Cottonseed, peanut, corn, soybean, sunflower seed, rapeseed (colza), sesame oil.

There are a number of oils, in addition to those mentioned above, which find a limited or occasional use as foodstuffs. Some of these oils, including teaseed, poppyseed, rice, almond, kapok, etc., are excellent edible products, but for one reason or another are not marketed in large quantities. Other oils, as, for example, linseed, are produced in considerable quantity, but have characteristics which make them more suitable for nonedible than for edible purposes.

2. World Production and Trade

The statistics relative to the production of edible oils are so generally inadequate that in many cases any estimate of world production can be regarded as little better than an intelligent guess. The best available sources of information, however, indicate that the average yearly world production during the period 1934–1938 of the more important edible oils was approximately as shown in Table 102.

TABLE 102

AVERAGE YEARLY WORLD PRODUCTION OF THE PRINCIPAL EDIBLE OILS AND FATS OF COMMERCE (1934–1938)

Oil	Quantity, 1000 million lbs.	Oil	Quantity, 1000 million lbs.
Butterfat	7	Whale oil	1.25
Lard	4	Palm oil	1.25
Cottonseed oil	3	Palm-kernel oil	0.75
Peanut oil	3	Corn oil	0.25
Coconut oil	2.5	Rapeseed oil	0.75[a]
Olive oil	2	Sunflower oil	0.75[a]
Soybean oil	1.75	Fish oils	0.50[a]
Tallow	1.5	Sesame oil	0.50[a]

[a] Uncertain, estimate very approximate.

In the preceding tabulation the figures include the entire production of each oil or fat, even though some of the production is used in nonfood fields. Much of the tallow produced is actually marketed in an inedible condition and is used for soap making. A large part of the coconut oil is also used for making soaps, and lesser quantities of the other oils are used for this and other nonedible purposes.

In considering the normal flow of oil and oil-bearing materials in the channels of world trade, a circumstance to be particularly noted is the large extent to which the great industrial nations, particularly those of Northern Europe, are dependent for these materials upon the nonindustrial regions of the tropics. Under normal conditions, not less than 6,000,000,000 lbs. of coconut, palm, palm-kernel, peanut, and cottonseed oils, either as such or in the form of oil seeds, are exported annually from Africa, India, British Malaya, the Netherlands East Indies, and the Philippines. Great Britain, Germany, France, the Netherlands, and the Scandinavian countries all rely heavily upon importations of these tropical oils. Even the United States, with a supply of 7,000,000,000 lbs. of edible oils and fats from domestic sources, imports three-quarters of a billion pounds of coconut and palm oils annually.

III. EXAMINATION AND EVALUATION OF OILS

1. Chemical Methods of General Applicability

Because of their relation to the fundamental factors of molecular weight and unsaturation in the fatty acids, the saponification value and the iodine value rank as the two most important characteristics in fat chemistry. The saponification value is defined as the number of milligrams of potassium hydroxide required to saponify one gram of fat. The same analytical results can be used for the calculation of saponification equivalent (grams of fat saponified by one gram molecular weight of KOH), or of the average molecular weight of the oil or its fatty acids. The iodine value is the percentage of iodine which the oil is capable of absorbing. There are a number of methods of determining the iodine value of which the Wijs method is to be generally preferred.

In the case of edible oils and their fatty acids the absorption of iodine is very nearly the theoretical amount, one diatomic molecule of iodine being added at each double bond. The thiocyanogen value (thiocyanogen absorbed, calculated in terms of iodine, on a percentage basis) is more difficult to determine than the iodine value, but is useful because thiocyanogen is principally added to but one of the double bonds of a di-unsaturated acid, and to but two of the three double bonds of a tri-unsaturated acid. This circumstance permits the composition of certain oils to be calculated directly from their iodine and thiocyanogen values. It was formerly believed that thiocyanogen, like iodine, was stoichiometrically added at each double bond with which it reacted, but recent investigations[1] have revealed that the actual absorption of thiocyanogen departs somewhat from the theoretical amount, and that empirical thiocyanogen values for the various fatty acids should be used in calculating the composition of fats or mixed fatty acids.

There are two methods in general use for effecting a more or less quantitative separation of saturated and unsaturated fatty acids. The Bertram oxidation method[2] probably gives the most accurate estimate of saturated acids, but has the disadvantage of destroying the unsaturated acids and making them unavailable for further analysis. In the Twitchell lead-salt alcohol method[3] and similar methods, the saturated acids are separated from the unsaturated acids by precipitation of their heavy metal salts in a suitable nonaqueous solvent. The two classes of acids can subsequently be easily regenerated after their separation as metallic salts. The separation is never completely quantitative, however, because of the slight solubility of the precipitated saturated acid soaps in the solvent, and the tendency of the precipitated soaps to

[1] R. W. Riemenschneider, C. E. Swift, and Chas. E. Sando, *Oil & Soap*, **18**, 203 (1941).

[2] K. A. Pelikan and J. D. Von Mikusch, *Oil & Soap*, **15**, 149 (1938).

[3] American Oil Chemist's Society, *Official and Tentative Methods.* Revised to January 1, 1941.

occlude small amounts of unsaturated acids. This method is generally applicable only to C_{16} and C_{18} acids.

Unsaturated fatty acids with three or more double bonds may be separated to some extent from the more saturated acids by virtue of the insolubility in cold ether of their bromine addition compounds. The separation is not quantitative, although the yield of insoluble bromides from an oil (its hexabromide number) is some indication of its content of linolenic or other highly unsaturated acids.

There are a number of characteristics which are indicative of the presence of low molecular weight fatty acids in an oil, and which were principally designed for detecting adulteration in butterfat. The Hehner value is the percentage of water-insoluble fatty acids. The Reichert-Meissl value indicates the content of soluble, steam-volatile acids (largely butyric, caproic, and caprylic). The Polenske value similarly indicates the insoluble, steam-volatile acids (largely capric and lauric). The Kirschner value is a relative measure of the butyric acid in a fat. The unsaponifiable matter in an oil comprises those components which do not form sodium or potassium soaps, but which are soluble in the common oil solvents. It usually consists largely of sterols, but will include any other nonfatty ether-soluble material, including mineral oil added as an adulterant.

The unsaponifiable matter of animal oils is characterized by the presence of cholesterol and that of vegetable oils by the presence of sitosterol. These two sterols and their compounds have different melting points and different crystalline forms, so that the examination of the unsaponifiable matter of an oil by suitable methods, as, for example, the digitonin-sterol acetate method, will identify it as animal or vegetable, or as a mixture of the two.

The fatty acids existing in an oil in a free state are estimated by titration of the sample with a standard alkali, in the presence of sufficient alcohol to serve as a solvent for the acids. The results are expressed as the acid value (mg. of potassium hydroxide to neutralize the free fatty acids in a gram of fat), or as the percentage of free fatty acids. In the latter case, the free acids are calculated as lauric acid for coconut and palm-kernel oils, and as oleic acid for most other oils.

2. Physical Methods of General Applicability

Fatty acids of different carbon chain lengths are readily separated by fractional distillation, in the manner to be outlined in a later paragraph. Because of their extremely low volatility (the vapor pressures of glycerides are of the order of one one-thousandth that of their fatty acids), glycerides cannot be distilled by ordinary methods. Their distillation can be effected, however, by the technique of molecular distillation.[4] Molecular distillation is very effective in concentrating sterols and other nonfat substances from natural oils, but does not effect fractionation of the glycerides to any very useful degree.

Fractional crystallization from solvents has been used to isolate specific glycerides from fats and oils, but the process is extremely laborious and the results are uncertain. Fatty acids of varying degrees of unsaturation are separated quite efficiently by low-temperature solvent crystallization,[5] although in some cases the formation of eutectic mixtures[6] limits the attainable degree of separation.

---

[4] Norris D. Embree, *Chem. Revs.*, **29**, 317 (1941).

Chromatographic adsorption methods[7] as applied to fats are a relatively new development, but they have been reported to be effective both in separating pigments and other nonoil constituents from oils, and in effecting separation of the different fatty acids.

The density of an oil is influenced both by the degree of unsaturation and the average molecular weight of its component fatty acids. The density increases with increased unsaturation, but decreases with an increase in the molecular weight of the acids. The differences in density between different oils is not large, however, and density or specific gravity determinations are of little or no value to the analyst. The density of a fat in the solid state is markedly greater than it is at the same temperature when liquid. For this reason, density determinations made on butter, lard, or other fats in the plastic state are more or less meaningless, since the density of such fats will to a great extent depend upon the highly indeterminate matter of their relative proportions of solid and liquid glycerides.

Natural fats, being complex mixtures, do not have true melting points, but melt gradually, over a considerable range of temperature. Most of the so-called "melting point" methods for fats actually determine the temperature at which the sample is partially melted, and at which it exhibits an arbitrary degree of softening. The Wiley melting point, the incipient melting point, the shot melting point, the slipping point, and the open tube melting point are all determined by methods in this category. Many fats exhibit the phenomenon of polymorphism and double melting point to a marked degree. Melting points by the conventional closed capillary method are sharp and likewise distinctive in the case of pure fatty acids and their simple mixtures. Fats which have been completely saturated by hydrogenation also melt sharply.

The solidification point, likewise known as the congeal point, or setting point is not definite for most natural fats, but is a useful characteristic in the case of certain hydrogenated fats. The solidification point of the mixed fatty acids, as determined by the titer test, is definite and is a fairly good indication of the amount of saturated acids in a natural oil, but not in a partially hydrogenated oil.

The refractive index of an oil depends directly upon its composition, the refractive index of the fatty acids increasing with increase in both the length of the carbon chain and the number of double bonds. By means of this characteristic, butterfat and coconut oil may be distinguished from ordinary high molecular weight fats or from each other. In the case of ordinary vegetable and animal oils, the refractive index is an indication of unsaturation, and can usually be correlated quite well with the iodine number. The refractive index has the great advantage of being among the easiest and quickest of the characteristics to determine. Chlorophyll, the carotenoids, and other pigments responsible for the color of oils may be identified and their concentrations judged from the absorption spectra of the oils.[7a]

---

[5] J. B. Brown and George G. Stoner, *J. Am. Chem. Soc.*, **59**, 3 (1937).

[6] H. W. Stewart and D. H. Wheeler, *Oil & Soap*, **18**, 69 (1941).

[7] Harold H. Strain, *Chromatographic Adsorption Analysis*. Interscience, New York, 1942.

In an exhaustive analysis of an oil it is often necessary to know whether an individual isolated fraction consists of a single fatty acid or of a mixture of fatty acids. This determination is a matter of more difficulty than might be supposed, owing to the marked tendency of certain fatty acids to form mixtures which behave like simple compounds in melting point, habit of crystallization, etc. The application of x-ray analysis to such fractions furnishes positive evidence as to their purity or lack of purity because of the characteristic crystal spacings of the different fatty acids.[8]

3. Determination of Fat Composition

As subjects for complete quantitative analysis, fats are among the most difficult of all substances. Because of their mixed nature, their limited chemical reactivity, and the close similarity of different members in physical properties, it is impossible to completely resolve fats into their component glycerides by any presently known method, although limited data may be obtained by the Hilditch[9] technique. Consequently, the composition of even the most common fats, in terms of their glycerides, is known in only a general way.

The analysis of a fat in terms of its component fatty acids is an easier matter, but one still beset with considerable difficulty. The technique usually employed involves the following successive steps: hydrolysis of the fat to yield its fatty acids in the free form; conversion of the acids to their methyl or ethyl esters (the esters are more stable and more easily distilled than are the free acids); separation of the esters into a number of close fractions by distillation under a vacuum, with the aid of an efficient fractionating column; and determination of average molecular weights, iodine and thiocyanogen values, and possibly also percentages of saturated and unsaturated acids, in the individual fractions. This procedure is quite effective in the analysis of oils like butterfat or coconut oil which have a diversity of saturated acids, but a limited number of unsaturated acids. As the vapor pressures of the fatty acids and their simple esters regularly increase with increase in the lengths of their carbon chains, each fraction will, under conditions of efficient fractionation, normally contain acids of not more than two different numbers of carbon atoms. The relative proportions of the two classes of acids may then be estimated from a determination of the average molecular weight of the entire fraction.

Distillation alone will effect no appreciable separation of fatty acids of the same chain length but different degrees of saturation, but fractions containing unsaturated acids may be separated into a saturated and an unsaturated portion, and the composition of the unsaturated portion may in most cases be estimated from its iodine and thiocyanogen values. The preceding method fails to completely identify the components of oils like whale and fish oils which have a large number of different unsaturated acids. In the case of such oils it is only possible for the analyst to report the percentage of unsaturated acids by groups (C_{16}, C_{18}, C_{20}, C_{22}, etc.) and the average number of double bonds contained in the acids of each group.

[7a] F. P. Zscheile, *Botan. Rev.*, 7, 587 (1941).

[8] Francis Francis, Harvey Piper, and Thomas Malkin, *Proc. Roy. Soc. London*, 128A, 214 (1930).

[9] T. P. Hilditch, *The Chemical Constitution of Natural Fats.* Chapman and Hall, London, 1940.

4. Tests for Specific Oils—Adulteration

Of the innumerable color tests which have been proposed for the detection of specific oils, only two, the Halphen test for cottonseed or kapok oil, and the Baudouin (or Villavecchia) test for seasame oil, are of outstanding reliability and usefulness. Either of these tests will positively detect as little as 1% of the proper oil in admixture with others. Hydrogenation of cottonseed oil makes it unresponsive to the Halphen test; the amount of hydrogenation required to destroy the test varies with hydrogenation conditions, but may be as little as 5 units in terms of reduction in iodine number. Hydrogenation of seasame oil does not destroy its reaction to the Baudouin test. Lard from hogs fed on cottonseed meal may give a slight Halphen reaction, and it is said that the milk fat of animals fed on sesame seed will at times give a positive Baudouin test. The Fitelson test for teaseed oil will detect as little as 5% of this oil in olive oil. All of the color tests depend upon the presence of characteristic nonoil substances. Except for those mentioned there are no reliable color tests for fats and oils.

Peanut oil differs from other common vegetable oils in its considerable content of arachidic acid, and other saturated fatty acids containing more than 18 carbon atoms. There are several tests for the presence of peanut oil which are based on the presence of these high molecular weight acids. Among them are the Renard, Bellier, and Kerr tests.

The Boemer test for the presence of beef fat in lard depends upon the difference in melting point of the α- and β-forms of monopalmitodistearin, and is the only example of a method of identification based on the presence of a specific glyceride.

In general, the detection and estimation of individual oils in mixtures will depend upon the good judgment of the chemist in the choice of his analytical methods and the interpretation of his results. In certain instances a few simple tests may indicate the composition of the product with a considerable degree of certainty. Thus a fat with a strong Baudouin test and a relatively low refractive index would certainly contain sesame oil and a considerable amount of either coconut or palm-kernel oil. In other cases, the most exhaustive analysis, extending even to a careful estimation of the component fatty acids, might fail to identify even a single component. A hydrogenated shortening or margarine, for example, made from any combination of cottonseed, soybean, corn, and sunflower oils would certainly be most difficult, if not impossible, to characterize.

The matter of adulteration of oil and fat products is of much less concern to food chemists today than it was 20 or 30 years ago. Regulatory measures covering labeling and adulteration are more common and more strictly enforced than they were formerly. In addition, modern oil-processing techniques have made the various oils and fats interchangeable to a greatly increased degree. The development of improved processing has had the effect of reducing former price differentials between different oils, and removing much of the incentive for adulteration. There was a time, for example, when the adulteration of lard with cottonseed oil or other vegetable oils was a matter of some concern. Today, these oils regularly sell at a price equivalent to, or greater than that of lard, and such substitution would be pointless.

It may be mentioned that oils which have been deprived of their characteristic odors and flavors by processing will in some cases again develop distinctive odors after they have been partially oxidized. Consequently, the odors developed by fatty products during the course of accelerated oxidation tests will often provide valuable clues to their composition. Palm oil and hydrogenated palm oil, when partially oxidized, acquire an odor reminiscent of violets. Soybean oil becomes first "beany" and then "fishy." Hydrogenated soybean oil develops a characteristic strawlike odor and taste. Both unhydrogenated and hydrogenated fish and whale oils tend to regain their original fishy odor. Unhydrogenated and hydrogenated rapeseed oils both acquire characteristic unpleasant odors.

5. Tests for Deterioration

A number of chemical tests have been devised for the detection of rancidity or the estimation of its intensity. Most of these are based upon either the aldehyde or the peroxide content of the rancid fat. The Kreis test, which is qualitative or roughly quantitative, depends upon the presence of a specific compound, namely, epihydrin aldehyde. The Schiff test is a qualitative test for aldehydes. The Schibsted method gives a quantitative estimation of high molecular weight aldehydes. The bisulfite method of Lea is intended to give an estimate of medium molecular weight aldehydes. The quantitative methods of Issoglio and Kerr depend upon the presence of low molecular weight, steam-volatile aldehydes. Peroxides are usually estimated iodometrically and reported in terms of peroxide value. For determining the peroxide value the techniques of Lea, Taffel and Revis, or Wheeler are most commonly used.

Many of the foregoing tests are both useful and reliable for the evaluation of fats of known composition and history. The oxidation reactions are so complex, however, and the concentration of any one class of reaction products is always so dependent upon the several factors of fat composition, rate of oxygen supply, presence or absence of accelerators and inhibitors, intensity of irradiation by light, temperature, etc., that none of these tests can be considered an absolute criterion, applicable equally to all fats under all conditions. Consequently, although the chemical tests may furnish strong corroborative evidence of the presence or absence of rancidity, there is as yet no means for its detection superior to the trained sense of smell of the experienced oil chemist.

6. Stability Tests

Methods for the detection of rancidity should not be confused with methods for determining the ability of oils to resist the development of rancidity. The laboratory tests for evaluating the stability or keeping quality of a fat are designed to accelerate the normal rate of oxidation sufficiently for the evaluation to be completed in a relatively short time, hence are collectively known as accelerated oxidation tests. The oxidation of a fat may be accelerated by raising its temperature, increasing the rate of oxygen supply to it, subjecting it to strong irradiation, or adding pro-oxidant substances to it. The course of oxidation may then be followed by measuring the oxygen absorbed, detecting or measuring the development of oxidation products chemically, or detecting the development of rancidity organoleptically. Numerous accelerated oxidation methods have been developed, and two, the Schaal test[10] and the so-called Swift stability test,[11] are in wide use.

The Schaal test involves a simple incubation of the oil sample in an open beaker at an elevated temperature (usually 60° C.). The keeping quality is expressed in terms of the number of days required to produce organoleptic rancidity. In the Swift test the sample is held at 208° F., and aerated at a rate sufficient to keep it saturated with air at all times. The keeping quality is expressed as the number of hours required for the sample to become rancid. With a given fat, organoleptic rancidity will be found to develop at a more or less specific peroxide value; consequently the personal factor may be eliminated from the test by means of a selected peroxide value as the end point. The values usually used, in terms of milliequivalents of peroxide per kg. of oil, are 20 for lard, 75 for hydrogenated vegetable oil shortening, 100 for vegetable shortenings of the compound or blended type, and 125 for cottonseed and similar oils. Keeping qualities in terms of hours by the Swift method are roughly equivalent numerically to keeping qualities in terms of days by the Schaal method.

The accelerated tests have proved extremely useful in many respects, but it should be recognized that they have distinct limitations. Their greatest utility has been as control methods for maintaining quality standards in the manufacture of lard and shortenings. In using them, attempts should not be made to interpret the test results too strictly in the case of samples of unknown history, and particular caution should be exercised in inter-comparing the results of tests on different varieties of fats. Accelerated oxidation necessarily follows a somewhat different course from normal oxidation, and the composition of the fat has an important bearing on the relative velocities of reaction in the two cases. If two samples of the same kind of fat exhibit a considerable disparity in stability under accelerated conditions, it may be expected that a more or less similar disparity will be found under normal storage conditions. A like correlation cannot be expected, however, if the fats are of different kinds, or if either contains added substances of a pro-oxidant or antioxidant nature.

The problem of stability evaluation is complicated by the fact that the keeping quality of packaged fats is generally of less practical concern than the keeping quality of the same fats after they have been incorporated into prepared foods. Fat stability is of particular importance in the case of crackers, biscuits, cookies, potato chips, and other products which are packaged and held for considerable periods of time before they are consumed. Because of the presence in such products of antioxygenic substances (which have an unequal effect on different fats), there is poor correlation between the stability of fats as such, and in the prepared products. The accelerated testing of the prepared products rather than the fats has been suggested, but the limited number of experiments thus far carried out[12] indicate the correlation between accelerated oxidation and normal oxidation to be even poorer in the case of these products than in that of the original fats. New and more reliable stability tests are urgently needed by the edible oil and fat industry.

---

[10] N. T. Joyner and J. E. McIntyre, *Oil & Soap*, **15**, 184 (1938).

[11] A. E. King, H. L. Roschen, and W. H. Irwin, *Oil & Soap*, **10**, 105 (1933).

7. Evaluation of Crude Oils

In refining crude oils with caustic soda, for the removal of their free fatty acids, there is a considerable loss of material. Obviously, the value of a given lot of oil will depend to a considerable extent upon the magnitude of the loss that must be incurred in processing it.

In order to establish an equitable system of evaluation for such oils, rather elaborate trading rules have been evolved based on a laboratory examination of the oils. In the United States practically all edible vegetable oils are bought and sold under such regulations. The original rules, as formulated by the National Cottonseed Products Association, were for cottonseed oil, but a similar system of oil grading is used by this and other trade organizations for soybean, peanut, corn, and coconut oils.

Under the above-mentioned rules a laboratory refining test is made, which more or less duplicates, on a miniature scale, the refining process as carried out in the plant. The sale price of the oil is then established on the basis of the yield of refined oil and its color, as measured on the Lovibond scale. In the case of cottonseed oil, a penalty amounting to three-quarters of one per cent of the contract price is assessed against the seller for each per cent that the refining loss exceeds 9%, and a similar premium is paid if the loss is less than 9%. A penalty of one-half of one per cent of the contract price is assessed for each unit of red color in the refined oil above 7.6 units, but no premiums are paid for color less than 7.6 red. There is an additional penalty of three-quarters of one per cent if the free fatty acid content exceeds 3.25%, or if the oil is off in flavor.

For evaluating refined oils, a laboratory bleach test is carried out. A standard fuller's earth is used as the bleaching agent. A bleachable cottonseed oil must have a color after bleaching not in excess of 2.5 red units.

8. Special Methods for Finished Products

A number of special methods are used for the examination of salad and cooking oils, margarines, shortenings, and other finished products. In all of these products, odor and flavor are highly important. The ability to properly judge the flavor of edible oils and fats is acquired only with experience and retained only with continued practice.

---

[12] R. H. McKinney and A. E. Bailey, *Oil & Soap*, **18**, 147 (1941).

The flavors and odors of butter, olive oil, and lard should be mild, but characteristic. In the case of deodorized oils and shortenings, standards vary to some extent in different places, but in the United States such products can be considered of the highest quality only if they are perfectly odorless and tasteless, and give only a sensation of oiliness in the mouth. The taste of margarine should be suggestive of butter, and not of the oils from which it is made.

The cold test (clouding time at 32° F.) is an indication of the suitability of an oil for mayonnaise manufacture. In shortenings which are to be used for baking, and also in margarine and other plastic products, the consistency of the product is a matter of paramount importance. Determinations of the melting point, titer, etc., are virtually useless as indications of this property. For a satisfactory evaluation of the body of a plastic product, the sample must be held for sufficient periods of time at each of several constant temperatures, and its consistency or plasticity determined at each temperature by penetration or other techniques.[13] If the determinations at the several temperatures are made in consistent units, a plasticity curve may be plotted, of plasticity against temperature. A set of temperatures commonly used is 50, 70, 80, 90, and 95° F. If a single portion only of the sample is available for examination, the successive plasticity determinations should be made at constantly increasing temperatures. For the plasticity examination of samples which have been melted, or for the examination of very small samples, the Freyer micropenetration technique[14] is suitable. Certain important physical properties of shortenings, including their ability to cream, or incorporate air, and to emulsify liquids, are best determined in an experimental bakeshop, using standard baker's mixing machines of small capacity.[15] The loaf volume produced by shortening in a standard pound cake formula, with no chemical leavening agent, is considered a particularly good index of its creaming qualities.

Shortenings of the superglycerinated or high-ratio type, which have mono- and diglycerides added to them to improve their baking and emulsifying properties, can be properly evaluated only by means of actual baking tests. The extent to which such a shortening has been superglycerinated may be determined from its glycerol content or from a quantitative estimation of its free hydroxyl groups, preferably after a concentration of the mono- and diglycerides by alcoholic extraction of the sample. The high-ratio shortenings are easily recognized by their low interfacial tensions against water, as determined by the ring method with a DuNouy instrument. The interfacial tension at 45° C. of an ordinary fat will be in the neighborhood of 26–28 dynes per cm.; that of a high-ratio shortening will usually be between 3 and 8 dynes.

If a fat is to be used for frying and particularly if it is to be so used in the home kitchen, its smoke point, or smoking temperature, is of some importance. A fat with a low smoke point may fume excessively at the high temperatures required for this method of cooking. In the case of fats used for commercial deep frying, however, there has been a tendency to exaggerate the importance of the smoke point. No fat retains an initially low smoke point with use, and under commercial frying conditions all fats tend to assume a common value, regardless of any original differences among them.[16] The smoke point of a fat depends almost entirely upon its content of free fatty acids, and varies inversely with the fatty acid concentration.[17]

---

[13] A. D. Rich, *Oil & Soap*, **19**, 54 (1942).

[14] Egbert Freyer, *Ind. Eng. Chem., Anal. Ed.*, **2**, 423 (1930).

[15] A. E. Bailey and R. H. McKinney, *Oil & Soap*, **18**, 120 (1941).

Partially hydrogenated fats may be recognized from their content of iso-oleic acids, which appear as unsaturated acids in the solid acid fraction by the Twitchell method. The iodine value of the solid acids of a natural fat will seldom exceed 4 or 5, as compared with 15–30 for most partially hydrogenated fats.

IV. CHARACTERISTICS

1. Composition of the Various Oils

The composition of any oil or fat is affected by a variety of biological and environmental factors. Consequently there are considerable variations in authentic samples

TABLE 103

COMPOSITIONS AND CERTAIN RELATED CHARACTERISTICS OF REPRESENTATIVE SAMPLES OF THE MORE COMMON HIGH MOLECULAR WEIGHT EDIBLE FATS AND OILS

Oil or fat	Iodine value	Refractive index at 60° C.	Sapn. value	Fatty acids, percentage by weight									
				Myristic	Palmitic	Stearic	Arachidic	C_{22} + C_{24} saturated	Palmitoleic	Oleic	Linoleic	Linolenic	C_{20} + C_{22} unsaturated
Linseed	180	1.4655	192	..	..	9[a]	..	..	..	23	20	48	..
Sardine	180	1.4654	189	5	15	3	..	..	12	10	15	..	40
Menhaden	170	1.4646	191	5	15	3	..	..	15	25	5	..	32
Safflower	145	1.4620	191	..	5	1	1	..	..	20	70	3	..
Poppyseed	134	1.4604	191	..	5	3	..	..	..	30	62	..	..
Soybean	130	1.4600	192	..	9	2	1	..	..	32	53	3	..
Sunflower	128	1.4597	191	..	5	2	1	..	..	35	57	0	0
Corn	125	1.4596	191	..	6	2	1	..	..	37	54	0	0
Whale	120	1.4590	194	5	15	3	..	..	15	35	10	..	17
Sesame	111	1.4582	192	..	8	3	1	..	..	47	41	0	0
Cottonseed	108	1.4572	195	1	21	2	1	..	..	25	50	0	0
Rapeseed	102	1.4582	175	..	1	1	..	1	..	22	22	3	50
Rice bran	98	1.4558	193	..	16	4	..	..	..	46	34	0	0
Kapok	98	1.4558	192	..	10	8	1	..	..	48	33	0	0
Peanut	90	1.4550	189	..	8	4	3	5	..	55	25	0	0
Teaseed	84	1.4542	192	..	8	2	..	..	..	82	8	0	0
Olive	83	1.4542	192	1	9	1	1	..	..	80	8	0	0
Lard (outer fat)	69	1.4526	195	1	26	6	..	..	3	52	12	..	..
Lard (leaf fat)	58	1.4513	195	1	28	8	..	..	2	56	5	..	..
Palm	53	1.4508	198	2	42	4	..	..	..	42	10	0	0
Tallow (beef)	45	1.4500	196	2	32	15	..	..	..	49	2	0	0
Tallow (mutton)	40	1.4494	196	2	34	19	..	..	..	43	2	0	0
Cocoa butter	36	1.4490	195	..	25	35	..	..	..	38	2	0	0

[a] Total saturated fatty acids.

---

[16] C. W. Lantz and G. T. Carlin, *Oil & Soap*, **15**, 38 (1938).

[17] D. A. Morgan, *Oil & Soap*, **19**, 193 (1942).

of the same type of oil. The compositions listed in Tables 103 to 108 are representative, however, and are believed to be somewhere near the average for normal samples from the regions where the respective oils are produced in the greatest quantity.

TABLE 104

COMPOSITIONS AND CERTAIN RELATED CHARACTERISTICS OF REPRESENTATIVE SAMPLES OF THE MORE COMMON LOW MOLECULAR WEIGHT EDIBLE FATS AND OILS

Oil or fat	Iodine value	Refractive index at 60° C.	Sapn. value	Fatty acids, percentage by weight										
				Butyric	Caproic	Caprylic	Capric	Lauric	Myristic	Palmitic	Stearic	Palmitoleic	Oleic	Linoleic
Butterfat	31	1.448	223	3	2	1	2	3	10	30	11	5	30	3
Palm-kernel oil (African)	17	1.443	245	0	..	3	7	47	14	9	1	..	18	1
Babassu oil	17	1.443	247	0	..	7	3	45	18	7	1	..	18	1
Cohune oil	10	1.441	252	0	..	7	7	45	18	9	3	..	10	1
Coconut oil	9	1.441	253	0	..	7	7	46	19	10	2	..	8	1

TABLE 105

MISCELLANEOUS CHARACTERISTICS OF THE MORE COMMON EDIBLE FATS AND OILS

Oil or fat	Density at 60° C.	Melting point, ° C.	Titer, ° C.	Unsaponifiable matter, %
Babassu	0.890–0.895	22–26	22–24	0.2–0.6
Butterfat	0.885–0.890	35–42	32–38	0.2–0.6
Cocoa butter	0.880–0.885	32–36	48–50	0.3–1.0
Coconut	0.890–0.895	22–26	20–24	0.1–0.3
Cohune	0.890–0.895	22–26	20–24	0.2–0.6
Corn	0.892–0.897	<0	18–20	0.5–3.0
Cottonseed	0.889–0.894	5–10	32–37	0.5–2.0
Kapok	0.888–0.893	5–10	25–30	0.5–2.0
Lard (outer fat)	0.882–0.887	30–40	32–37	0.2–0.4
Lard (leaf fat)	0.881–0.886	44–48	39–42	0.2–0.4
Linseed	0.902–0.907	<0	14–16	0.5–1.5
Menhaden	0.900–0.905	<0	24–28	0.5–1.5
Olive	0.885–0.890	0–10	18–25	0.5–2.0
Palm	0.882–0.887	25–30	40–45	0.2–0.6
Palm-kernel (African)	0.890–0.895	22–26	22–24	0.2–0.5
Peanut	0.885–0.890	5–10	28–32	0.4–1.0
Poppyseed	0.896–0.901	<0	16–18	0.5–1.0
Rapeseed	0.880–0.885	<0	11–15	0.5–1.5
Rice bran	0.888–0.893	0–10	23–28	0.5–4.0
Safflower	0.898–0.903	<0	15–17	0.5–1.5
Sardine	0.902–0.907	<0	24–28	0.5–1.5
Sesame	0.889–0.894	<0	22–26	0.5–1.5
Soybean	0.895–0.900	<0	20–32	0.5–1.5
Sunflower	0.894–0.899	<0	17–20	0.3–0.5
Tallow (beef)	0.881–0.886	40–50	36–46	0.2–0.4
Tallow (mutton)	0.881–0.886	44–50	40–48	0.2–0.4
Teaseed	0.885–0.890	5–10	15–20	0.5–1.5
Whale	0.890–0.895	0–5	18–25	0.8–3.0

TABLE 106

SPECIAL CHARACTERISTICS FOR THE DETECTION OF ADULTERATION IN BUTTERFAT

Fat	Reichert-Meissl value	Polenske value	Kirschner value
Butterfat	22–32	2–4	20–25
Coconut oil	6–8	14–16	1–2
Palm-kernel oil (African)	5–7	10–12	0.5–1
Babassu oil	5–7	10–12	0.5–1
Cohune oil	6–8	14–16	1–2
Other common oils	<1	<1	<0.5

TABLE 107

CHARACTERISTICS OF CERTAIN COMPLETELY HYDROGENATED EDIBLE OILS

Oil or fat	Refractive index at 60° C.	Melting point, ° C.	Titer, ° C.
Coconut oil	1.4400	44.8	42.0
Corn oil	1.4470	69.0	65.0
Cottonseed oil	1.4462	62.0	61.5
Sardine oil	1.4464	60.0	55.0
Sesame oil	1.4466	68.0	66.0
Soybean oil	1.4464	68.0	65.5
Sunflowerseed oil	1.4472	69.5	65.0
Tallow, beef	1.4456	61.0	60.5

TABLE 108

MISCELLANEOUS PHYSICAL PROPERTIES COMMON TO VARIOUS FATS AND OILS

(Refined oils composed largely of C_{18} acids, as cottonseed oil, soybean oil, lard, etc.)

Smoke point, ° F. (0.02% free fatty acid)		440–450
Flash point, ° F. (0.02% free fatty acid)		615–625
Fire point, ° F. (0.02% free fatty acid)		675–685
Specific heat (average, at processing temperatures)		*ca.* 0.5
Viscosity (centipoises)	at 20° C. (68° F.)	*ca.* 48
	at 60° C. (140° F.)	*ca.* 18
	at 100° C. (212° F.)	*ca.* 7.5
Solubility of oxygen[a]	at 20° C. (68° F.)	0.125
	at 60° C. (140° F.)	0.142
	at 150° C. (302° F.)	0.182
Solubility of nitrogen[a]	at 20° C.	0.067
	at 60° C.	0.083
	at 150° C.	0.119
Solubility of hydrogen[a]	at 20° C.	0.039
	at 60° C.	0.059
	at 150° C.	0.104
Solubility of water[b]	at 0° C. (32° F.)	0.00074
	at 20° C. (68° F.)	0.00115
	at 35° C. (95° F.)	0.00145
Change in density with temperature		0.000660 per ° C.
		0.000367 per ° F.
Change of refractive index with temperature		0.00038 per ° C.

---

[a] Volumes of gas which are soluble in a unit volume of oil.

[b] Weight of water dissolved by a unit weight of oil.

2. Characteristics of the Various Oils

What has been said above with regard to the composition of the various oils applies equally to their related characteristics. Certain of these characteristics, namely, the iodine value, refractive index, and saponification value, are so directly related to the composition of the oil that it is preferable to list representative and mutually consistent values for these characteristics also, rather than a range of values. No information is, therefore, given relative to the variations to be expected in different lots of the same kind of oil, but this procedure is considered preferable to the confusion which is generally created by listing the full range of possible normal or abnormal variations. The iodine number of outer fat lard, for instance, is given as 69, since it is an average value for lard from the corn-hog belt of the United States. A range of iodine values of 45 to 55 might be quite normal, however, for European lard from hogs fed on a high portein diet, and a range of 80 to 90 might be normal for hogs fed on peanuts.

3. Other Characteristics

Linseed oil, and menhaden, sardine, and other fish oils are generally considered inferior for edible purposes because of their high degree of unsaturation. In addition, fish oils are often rendered from badly damaged materials, which contributes to their high free fatty acid content and generally poor quality. Whale oil is usually rendered from freshly killed animals, in modernly equipped factory whaling ships. After hydrogenation it is entirely suitable for use in edible products, although it is susceptible to some degree of flavor reversion.

Soybean oil is subject to a slight flavor reversion, which in the past has influenced the price so that it sold slightly under that of cottonseed and certain other vegetable oils. Hydrogenated soybean oil is more stable toward rancidity than hydrogenated cottonseed oil. Crude soybean oil is characterized by a considerable content of phosphatides and is the richest commercial source of lecithin. Safflower oil is very similar in characteristics to high iodine value soybean oil.

Sunflowerseed, corn, and poppyseed oils are all quite similar. All are excellent edible oils, although poppyseed oil is somewhat less stable than the other two. Corn oil is characteristically somewhat dark in color. Cottonseed oil is the standard American all-purpose edible oil. In its crude form it has a considerably higher content of nonoil substances than most other oils. Peanut oil is suitable for all purposes for which cottonseed oil is used, except the manufacture of winterized salad oil, and has the advantage over cottonseed oil of being more stable and lighter in color. Sesame oil is highly prized for use in edible products because of its potent content of antioxidants, which are fully activated, however, only by hydrogenation of the oil. Hydrogenated rice bran oil is also extremely stable, although somewhat dark in color. Kapok oil is quite similar to cottonseed oil.

Rapeseed oil is very difficult to deodorize and has an unpleasant flavor reversion, hence it is not considered a prime edible oil. Olive oil is the standard edible oil of Southern Europe, and the Mediterranean region. Chemically, it is unique in its high content of oleic acid, which causes it to be low in both melting point and iodine value. Teaseed oil is the only oil produced in quantity with a composition approaching that of olive oil.

Palm oil is characterized by a very high content of carotene, which colors it a deep orange-red. In spite of improvements in the process of extracting palm oil in recent years, this oil is still marketed with a free fatty acid content of 3–10%, which is much greater than that of most other vegetable oils. The dark red color of palm oil is readily bleached to a light yellow by hydrogenation.

Lard and butterfat are the traditional edible fats of Northern Europe. Butter is the only important source of vitamins among the various edible fat products. It contains considerable amounts of vitamins A and D. Lard is extremely low in its content of nonoil substances, and thus it is probably the purest of all the natural fats and oils. Its high purity and consequent lack of antioxygenic constituents make it relatively unstable. The iodine value of lard varies widely, according to the portion of the carcass from which the fat is taken and the feed of the animal. Leaf or kidney fat has the lowest, and back fat the highest iodine value.

Beef tallow and mutton tallow are very similar in properties and the two are often mixed at meat-packing plants. Much beef tallow is separated into oleostearine and oleo oil by partial crystallization and pressing, although the demand for these two products has declined since the introduction and general use of the process for hydrogenating vegetable oils. Oleo stock is the select grade of tallow from which oleostearine and oleo oil are prepared.

Cocoa butter is used almost entirely as a confectionery fat, being particulary suited to this purpose by virtue of its relatively low melting point combined with a brittle consistency at ordinary temperatures.

Coconut, palm-kernel, babassu, and cohune oils are all very similar. They are good fats for the manufacture of margarine and confectionery, but because of their combination of low melting point with brittleness at low temperatures, and their tendency to foam in deep frying, they are not suited for plastic shortening manufacture without extensive admixture with other fats.

V. UTILIZATION

1. Variations in Consumer Taste

The edible oils and fats may be divided into two broad classes, according to whether their natural odor and flavor components are considered pleasing and desirable, and whether they are removed or partly removed before the oil is consumed.

There is a considerable overlapping of the two classes, due to local variations in custom and taste. In the less highly industrialized regions of the world even some of the more strongly flavored oils are largely consumed in the crude state. The opposite extreme obtains in certain sections of the United States, where consumers are inclined to demand strict neutrality of flavor in all of their edible oils, with the exception of butterfat. In regions where much margarine is consumed, neutral fats are in general demand, as this product requires a flavorless oil in its manufacture. In general, it may be said that olive oil, butterfat, and lard are the important fats of the first class, as they are marketed everywhere without being processed for flavor removal. Cottonseed oil is decidedly in the second class, since it is so strongly flavored that it is virtually inedible without drastic processing treatment. Practice varies to some extent with regard to peanut, soybean, sunflower, sesame, and most of the other seed oils. In the Orient these oils are commonly consumed with little or no treatment. In most parts of the Western world, however, these oils belong to the second class, and are usually deodorized and consumed in a more or less odorless and tasteless form.

2. Variations in Culinary Custom

From the earliest times, the peoples of Africa, Southern Asia, and Southern Europe have derived their edible oils primarily from vegetable sources, whereas Northern Europeans have obtained their fats from domestic animals. This circumstance has resulted in a decided difference in habits of fat utilization between Northern and Southern European people and their descendants throughout the world.

Southern European cooking is adapted to the use of olive oil and other liquid oils. That of Northern Europe is built around the use of the plastic fats such as butter and lard. For many purposes, liquid oils and plastic fats are not interchangeable. It would not be possible, for instance, to prepare a salad with lard or butter, or to make a pound cake with olive oil.

During the past century there have been vastly increased plantings of oil-bearing seeds in the temperate regions of the globe and, in addition, the vegetable oils of the Orient have been exported to Europe and the Americas in increasing quantities. As a consequence, many countries which formerly consumed animal fats almost exclusively now have a large per capita consumption of edible vegetable oils. To a large extent, however, these vegetable oils are converted by hydrogenation and other processing treatments to margarine and shortening, which have the plastic properties, respectively, of butter and lard.

3. Shortening Agents

Shortening agents are distinguished by their plasticity, which enables them to form with milk, flour, etc., the peculiar dough structure which is essential for the production of good baked products. The original shortening agent was lard. The development of vegetable shortenings resulted from the necessity of finding an outlet for the large North American production of cottonseed oil. The first vegetable shortenings were primarily products of a lardlike consistency prepared by blending cottonseed oil with oleostearine; later the product was more commonly stiffened by hydrogenation of the cottonseed oil. These early vegetable oil shortenings were admittedly only lard substitutes. Continued improvement in the technology of vegetable shortening manufacture has, however, resulted in a product which is superior to lard in many respects, and which has in late years commanded a consistently higher price on the market.

Since cottonseed oil has a naturally unpleasant taste, the manufacturers of vegetable shortenings of good quality have adopted the practice of rendering their products completely neutral in flavor. As a consequence, a considerable part of the consuming public appears to have developed a disaffection for natural fat flavors, and thus the distinctive flavor of lard has become in some quarters a liability rather than an asset. The qualities in which lard is particularly inferior to good vegetable shortenings are its stability, and its ability to emulsify air and liquids in the preparation of baked goods. Vegetable shortenings are consequently preferred for the manufacture of packaged products and for cake making. Lard, being softer, is somewhat superior in shortening power to vegetable shortenings, and is often preferred for making bread and pie crusts. In the Southern United States, lard is in some demand as a seasoning agent for cooking vegetables, etc. In recent years, lard producers have to a limited extent begun to apply the processing treatments of refining, hydrogenation, deodorization, etc., to improve the quality of their product. They have also experimented, with the addition of gum guaiac and other nontoxic antioxidants, to compensate for the natural lack of such substances in lard.

Attempts have been made to improve the uniformity and general quality of lard by the establishment of more rigid standards governing its production. Recently promulgated regulations of the U. S. Department of Agriculture require substandard stock to be rendered and marketed as rendered pork fat.

Vegetable type shortenings may be of the compound or blended variety, or the all-hydrogenated variety. In the manufacture of the former, the product is stiffened to the proper consistency by the addition of a small proportion of relatively hard material, such as oleostearine, tallow, or highly hydrogenated oil. All-hydrogenated shortenings are stiffened by hydrogenating the entire body of oil to the proper consistency. They have a lower iodine value and a greater stability than blended shortenings, are generally processed with more care, and are sold at a higher price. All-hydrogenated shortenings are of the standard, all-purpose type, the biscuit and cracker type, or the high-ratio or superglycerinated type. In the hydrogenation of shortenings of the biscuit and cracker type, the stability of the product is improved at some expense to

TABLE 109

CHARACTERISTICS OF VARIOUS TYPES OF SHORTENING

Analysis	Blended shortening	All-hydrogenated shortening	Prime steam lard
Iodine value	90	62	67
Stability: Swift method (hrs.)	20	75	10
Free fatty acids (% as oleic)	0.12	0.02	0.30
Color (Lovibond scale)	20Y-2.5R	10Y-1.2R	5Y-0.8R
Flavor and odor	Slight	None	Characteristic
Plasticity: (by the Freyer micropenetration method)			
at 50° F.	30	20	50
at 70° F.	75	60	110
at 80° F.	140	115	160
at 90° F.	185	215	250
at 95° F.	230	300	400

its plastic range. High-ratio shortenings are similar to those of the standard type except that they have added to them in the neighborhood of 5% of mono- and diglycerides, to improve their baking and emulsifying properties. Large quantities of plastic shortenings are also used for deep frying and other purposes for which a plastic product is not strictly necessary.

Analyses of typical samples of blended shortening, standard all-hydrogenated shortening, and prime steam lard are shown in Table 109.

4. Butter and Margarine

Margarine was invented during the Franco-Prussian war by the French chemist Mège-Mouriès, in response to a contest sponsored by Napoleon III to develop a butter substitute. Margarine is prepared by blending animal or vegetable fats of a butterfat-like consistency with salt and properly cultured and ripened skim milk. Its flavor is not due simply to the lactic acid of the milk but also to odor and flavor producing substances which are developed by contact of the milk with the fat. The flavor of good margarine is very similar to, but not identical with that of butter. The manufacture of butter and its characteristics are discussed in Volume II, Chapter XX.

Margarine, like shortening, is essentially a plastic product, but the consistency of margarine is in some respects more critical than that of shortening. In order that it may have a body resembling that of butter, and in order that it may be easily formed into the blocks or prints in which it is customarily marketed, it must be quite firm at temperatures of 35–45° F. At room temperatures it must spread readily, but it must melt easily and completely at 98° F. or less, in order that it will not give the effect of pastiness in the mouth.

Preference in the matter of fats for margarine manufacture has passed through three successive stages. The original margarine fat was beef fat. Oleo oil has body characteristics quite close to those desired, and was for many years considered the ideal margarine fat. Later, coconut, palm-kernel, and similar oils largely displaced oleo oil. In recent years, the tendency has been for margarine manufacturers to rely upon the hydrogenation process to obtain the desired body in their product. This practice has made possible the use in large quantities of such oils as cottonseed, soybean, and whale oil (see Table 110).

Margarine is manufactured in huge quantities in Europe, but its production in the United States has been much restricted by discriminatory legislation, including a federal tax of 10 cents per lb. on yellow-colored margarine. In a number of states, taxes and other restrictions virtually prohibit its manufacture, but lately there has been a tendency to liberalize antimargarine legislation. A recent ruling of the Food and Drug Administration enables margarine manufacturers to remedy the principal deficiency of their product as compared to butter by incorporating in it vitamins from outside sources.

Several types of margarines are made for special use by commercial bakers. These include puff paste, a stiff waxy product of high melting point, for use in making Danish type pastry. Puff paste may be made by blending the fat with water instead of milk,

TABLE 110

OILS AND FATS USED IN THE MANUFACTURE OF SHORTENING AND MARGARINE IN VARIOUS COUNTRIES IN RECENT YEARS (MILLIONS OF POUNDS)

Oil or fat	U. S. (1941)		United Kingdom (1937)		Germany (1935) Shortening and combined margarine[a]
	Shortening	Margarine	Shortening	Margarine	
Cottonseed oil	889	150	87	31	..
Peanut oil	82	2	49	69	29
Soybean oil	216	76	7	0	60
Palm-kernel oil	0	1	2	22	126
Coconut oil	22	30	0	60	99
Palm oil	86	5	13	..	..
Whale oil	0	0	67	148	470
Fish oils	6	0	0	..	..
Tallow and tallow derivatives	65	21	4	16	7
Pork fat	51	8	0	2	4
Miscellaneous oils	1	2	10	11	69
Total	1418	295	239	359	864

[a] Approximately 90% margarine and 10% other edible fats.

and due to its high melting point is not legally classified as a margarine. The fat is usually composed of about 35% oleostearine and 65% cottonseed oil. There are a number of cake maker's margarines, etc., with properties intermediate between those of puff paste and ordinary table margarine.

5. Salad and Cooking Oils

A cooking oil may be any edible oil, but a salad oil other than olive oil is generally understood, at least in the United States, to be one which will not solidify at ordinary domestic refrigerator temperatures of 40–45° F. Such an oil will not only pour from its container when cold, but also will not crystallize and break the emulsion of mayonnaise type salad dressings. Natural cottonseed oil solidifies at the above-mentioned temperatures, but can be winterized to produce an oil that does not, by removal of its higher melting portions. Corn oil is a natural winter oil, and most of the corn oil produced in the United States is sold as a salad oil. Soybean oil requires the removal of a very small quantity of wax from it to prevent its clouding at low temperatures, but sunflower oil and sesame oil are both natural winter oils. Peanut oil cannot be winterized.

6. Specialty Oils and Fats for Special Purposes

A number of specialty uses for different oils and fats may be mentioned. The use of cocoa butter as a confectioner's fat has been mentioned previously. The best substitute for cocoa butter is a stearine from an oil of the coconut type. Enrobing fats, for the manufacture of filled biscuits, etc., are usually composed largely of hydrogenated coconut oil. Because of its flavor, undeodorized tallow is in demand for frying hamburgers, etc. Coconut oil is sprayed on the surface of a certain type of crackers, to impart flavor to the crackers and luster to their surface. Solid shortenings, consisting of a highly hydrogenated vegetable oil, are used in the preparation of some prepared cake mixes, biscuit mixes, etc. A small amount of unbleached palm oil has been used to impart a natural yellow color to margarine. Lard oil (lard with its high melting point portions removed by pressing) is used by commercial bakeries for greasing bread pans. Considerable quantities of liquid oils, particularly cottonseed oil, are used for packing sausages, sardines, etc.

Selected Bibliography

General Chemistry and Technology, including methods of analysis:

Dean, H. K., *Utilization of Fats.* Chemical Pub. Co., New York, 1938.
Hefter, G., and Schonfeld, H., *Chemie u. Technologie der Fette u. Fettprodukte.* Springer, Vienna, 1936–1939.
Hilditch, T. P., *Industrial Fats and Waxes.* Bailliėre, Tindall and Cox, London, 1941.
Jamieson, George S., *Vegetable Fats and Oils.* 2nd ed., A. C. S. Monograph No. 58, Reinhold, New York, 1943.
Lewkowitsch, J., and Warburton, G. H., *Chemical Technology of Oils, Fats and Waxes.* 6th ed., Macmillan, London, 1921.

Composition of Oils and Fats:

Hilditch, T. P., *The Chemical Constitution of Natural Fats.* Chapman and Hall, London, 1940.

Deterioration of Oils and Fats:

Lea, C. H., *Rancidity in Edible Fats.* Chemical Pub. Co., New York, 1939.

Production and Trade:

Imperial Economic Committee, *Vegetable Oils and Oilseeds.* H. M. Stationery Office, London, 1938.
Internatl. Inst. Agr., *Oils and Fats: Production and International Trade. Parts I and II.* Rome, 1939.
U. S. Dept. Agr., *Agricultural Statistics,* U. S. Government Printing Office, Washington (1942 and previous years).

Methods of Analysis and Trading Rules:

American Oil Chemist's Society, *Official and Tentative Methods.* Revised to January 1, 1941.
Assoc. Offic. Agr. Chem., *Official and Tentative Methods of Analysis.* 5th ed., Washington, 1940.
Natl. Cottonseed Prods. Assoc., "Rules Governing Transactions between Members." Adopted May, 1943.

Chapter XVII

CEREAL GRAINS*

W. F. GEDDES
St. Paul, Minnesota

I. HISTORY, PRODUCTION, STRUCTURE, CLASSIFICATION, AND STORAGE PROPERTIES

1. Introduction

Under extensive systems of agriculture such as that of the United States, the important field crops belong chiefly to two botanical families, the *Gramineae,* or grass family, and the *Leguminosae,* or legume family. The grass family comprises many thousand species and includes the majority of the cultivated forage crops as well as those crops which are grown mainly for their grains and which supply the bulk of the food consumed by the human race. Members of the grass family which are grown for their edible grains are called cereals or cereal grains, the most important of which are wheat, rye, barley, maize (corn), oats, and rice; the sorghums are employed principally for animal feeding. Buckwheat is not a true cereal since it is not a member of the grass family. However, it is similar in composition and use to the cereal grains, and from the standpoint of the food technologist it is convenient and logical to consider it along with the cereal grains. The botanical classification of the economically important cereals grown in the United States is given in Table 111.

The cereal grains are, in general, the cheapest sources of food energy and they normally constitute one-third and more of the calorific and protein intake of humans.

Their prominence as food plants is due to their adaptability to widely varying soil and climatic conditions, their high yielding ability, their ease of cultivation with a small amount of labor, their excellent storage properties, and their high food value. Wheat is the chief cereal of the temperate regions; barley and rye can be grown advantageously in the more northerly regions while maize and rice are particularly suited for cultivation in the tropics and the warmer temperate areas. Grain sorghum, like maize and rice, is a "tropical" cereal but is better suited than corn to cultivation under semiarid conditions.

The cereals have been known for many thousands of years, and wheat, maize, and rice, in particular, have played an important part in the development of civilization.

---

* This chapter is closely allied to Chapter XV of Volume II.

They were grown by primitive peoples before any recorded history of man so that the wild forms from which they were evolved are not known and their origin is obscure. Maize is the only cereal which is indigenous to the Western Hemisphere. It was found under cultivation when Columbus discovered America and, soon after, was introduced into the Eastern Hemisphere by the early explorers. Wheat, oats, barley, and rye, according to Hutcheson, Wolfe, and Kipps,[1] are not believed to have been cultivated in America prior to its discovery by Columbus and production was started by the colonists along the Atlantic coast early in the 17th century. Buckwheat was also grown by the early colonists, but rice was introduced somewhat later, being first grown in South Carolina in 1694. The sorghums were introduced much later and did not become an important crop until the middle of the last century. During the long period of cultivation of these grain crops throughout the world, new species and varieties have been evolved. With the introduction of purposeful plant breeding, which has been an important feature of American agriculture, marked advances have been made in the development of varieties which are better adapted to various cultural conditions or which are of higher quality for specific purposes.

TABLE 111

BOTANICAL CLASSIFICATION OF COMMON GRAINS GROWN IN THE UNITED STATES

Common name	Family	Tribe	Genus	Species	Subspecies
Barley:					
Two-row	*Gramineae*	*Hordeae*	*Hordeum*	*distichon* L.	....
Six-row	*Gramineae*	*Hordeae*	*Hordeum*	*vulgare* L.	....
Buckwheat	*Polygonaceae*	*Polygonoideae*	*Fagopyrum*	*escutentum* Moench.	....
Corn or maize:					
Dent corn	*Gramineae*	*Maydeae*	*Zea*	*mays*	*indentata* Sturt.
Flint corn	*Gramineae*	*Maydeae*	*Zea*	*mays*	*indurata* Sturt.
Popcorn	*Gramineae*	*Maydeae*	*Zea*	*mays*	*everta* Sturt.
Sweet corn	*Gramineae*	*Maydeae*	*Zea*	*mays*	*saccharata* Sturt.
Oats:					
White and yellow	*Gramineae*	*Aveneae*	*Avena*	*sativa* L.[a]	....
Red	*Gramineae*	*Aveneae*	*Avena*	*byzantina* L.	....
Rice	*Gramineae*	*Oryzeae*	*Oryza*	*sativa* L.	....
Rye	*Gramineae*	*Hordeae*	*Secale*	*cereale* L.	....
Wheat:					
Club wheat	*Gramineae*	*Hordeae*	*Triticum*	*sativum*	*compactum* Host
Common wheat	*Gramineae*	*Hordeae*	*Triticum*	*sativum*	*vulgare* Vill.
Durum wheat	*Gramineae*	*Hordeae*	*Triticum*	*sativum*	*durum* Desf.
Sorghum, grain	*Gramineae*	*Andropogoneae*	*Andropogon*	*sorghum* Brot.	....

[a] Includes *Avena orientalis* Schreb, or Tartary oats, which have one-sided panicles.

2. Production

The importance of the cereal crops in American agriculture is indicated by the average acreage and production figures for the years 1930–1939, given in Table 112.

---

[1] T. B. Hutcheson, T. K. Wolfe, and M. S. Kipps, *The Production of Field Crops*. McGraw-Hill, New York, 1936.

Corn is the leading grain and the U. S. production represents nearly 50% of the estimated total world production. Oats, wheat, and barley follow corn in production volume while rice, rye, and buckwheat are relatively minor crops.

TABLE 112

AVERAGE U. S. PRODUCTION OF SELECTED GRAINS—1930 TO 1939, INCLUSIVE[a]

Grain	Acreage, 1000 acres	Yield per acre, bu.	Production, 1000 bu.	Percentage of estimated world production, %
Barley	10,707	20.6	224,970	9.8
Corn[b]	98,049	23.5	2,307,452	49.4
Oats	36,487	27.3	1,007,141	22.8
Rice, rough[c]	942	48.4	46,563	....
Rye	3,320	11.2	38,472	0.4 (exclusive of China and U. S. S. R.)
Sorghums, grain	7,564	11.0	84,253	....
Wheat	55,884	13.3	747,507	19.1 (exclusive of China and U. S. S. R.)
Buckwheat	460	16.0	7,315	....

[a] Compiled from data given in *Agricultural Statistics, 1941* (U. S. Dept. Agr.) and *Northwestern Miller*, Almanac No. 210 (5), (April, 1942). The bushel weights adopted by the Bureau of Statistics, U. S. Dept. Agr. are: barley, 48 lbs.; buckwheat, 48 lbs.; corn (shelled), 56 lbs.; oats, 32 lbs.; rice (rough), 45 lbs.; rye, 56 lbs.; sorghum, 50 lbs.; and wheat, 60 lbs. The standard bushel (Winchester bushel) in the United States contains 2150.42 cu. in., while the English (Imperial bushel) is 3.1% larger and contains 2219.36 cu. in.

[b] U. S. figures include production for all purposes, including hogged and siloed corn and that cut and fed without removing the ears as well as that husked and snapped for grain; the production is calculated on the basis of the total acreage being harvested as grain.

[c] World production figures are in terms of milled rice; the average U. S. production of milled rice for 1930–1939 was 1269 million lbs. or 0.9% of the estimated world production exclusive of China.

(*a*) *Barley*

Barley[2] is one of the prehistoric cereal grains used for food, feed, and fermented beverages. Excavations in Egypt, Mesopotamia, and other ancient centers of civilization in the eastern hemisphere indicate that barley, like wheat, has had an important role in Asiatic and European development. Both the two-rowed (*Hordeum distichon* L.) and the six-rowed (*H. vulgare* L.) and wild barley were represented in those prehistoric forms. Representatives of the two species are well distributed today with a continued general tendency for the naked barley (kernels separate from the hulls in threshing) to predominate in areas where barley is used largely for human food and hulled barleys where used for fermented beverage production and feed for livestock.

The original introductions of barley into the United States were from Europe and North Africa. The first barleys grown in the various American settlements undoubtedly originated from the homes of the early colonists. The English probably brought with them the two-rowed barleys such as Chevalier and Thorp. The Dutch introduced the varieties from the continent. The Spanish brought the barleys of the coast type (probably originating from North Africa); these types have always predominated in the southwestern United States. The Continental European barleys, especially the six-rowed varieties, were found to be well adapted to the northeastern and northcentral United States. The reports of the Albany, N. Y., grain trade in the 1850's show that the six-rowed barleys were more common than the two-rowed; the Manchurian types were introduced from Germany into Canada and Wisconsin about 1873 and soon became the predominating barleys in the spring barley area of the United States and Canada. The Manchurian-Oderbrucker type has predominated in this area until the development of the hybrid smooth-awned varieties during the past decade. The early production of barley in North America was concentrated around the malting and brewing centers located in New York, Pennsylvania, Ohio, Illinois, Wisconsin, and Minnesota and on the Pacific coast. Since 1911 barley acreage has expanded from these centers as it became an important feed grain, along with oats and corn, in the production of livestock.

---

[2] Contributed by J. G. Dickson, Professor of Plant Pathology, University of Wisconsin.

As shown in Table 112, barley ranks third among the small grains in volume of production in the United States; the major production is in the northcentral spring barley area and the southwest coast type area. In Canada the production is largely in the spring barley area contiguous with the northcentral area of the United States.

(*b*) *Buckwheat*

Buckwheat, a small branching annual with smooth succulent stems, is essentially a plant of cool, moist, temperate regions, which is planted late in the spring to avoid frosts; it is well adapted to cultivation on poor soils. It is grown chiefly in the States of Pennsylvania and New York, and while it is a relatively minor crop, as compared with the true cereals, the United States ranks next to Russia and France as a buckwheat-producing country. The principal varieties are Silverhull and Japanese, the former being preferred for milling purposes because of its higher endosperm yield.

(*c*) *Corn*

Corn requires warm, moist growing conditions and its principal commercial production is in the so-called "corn belt," which includes all of Iowa and Illinois and parts of Indiana, Kansas, Kentucky, Michigan, Minnesota, Missouri, Nebraska, Ohio, South Dakota, and Wisconsin. In this region the dent type (*Zea indentata*) is predominant; dent corn constitutes more than 90% of the entire U. S. production. The production of flint corn, which is early maturing, is principally confined to northern Pennsylvania, New York, the New England States, California, and to the western and northern edges of the corn belt. Sweet corn (*Z. saccharata*) is adapted to the cooler areas and is the type grown for canning purposes for which the grain is used in the unripe state; the North Atlantic and Central States are important producers of sweet corn. In recent years, marked improvements in the yield and quality of corn for specific purposes have been made by the widespread culture of hybrid corn (the first generation hybrid between two or more inbred strains).[8] Certain varieties of corn, as well as of sorghum, millet, barley, and rice, yield so-called waxy starch which stains a reddish-brown rather than a blue color with iodine. Waxy starch has physical properties which correspond rather closely to those of cassava, the root starch from which tapioca is prepared. Hixon and Sprague,[4] in reviewing the history, production, and properties of waxy starch, have pointed out that of the different waxy cereals, corn appears to offer the best possibilities as a commercial source since the waxy character can be readily introduced into hybrids which are similar in other particulars to ordinary hybrid corn now produced and processed on a large scale.

---

[8] A description of the method of producing hybrid corn is given by F. D. Richey, "The What and How of Hybrid Corn," U. S. Dept. Agr., *Farmers Bull.* **1744** (1935).

(*d*) *Oats*

In volume of production, oats are the second leading cereal of the United States and for the ten-year period 1930–1939, they comprised 43.6% of the tonnage of corn. While they are grown throughout the United States, the chief producing areas conform somewhat closely to the North Central "corn belt" but extend farther northward. Of the average production of 1,007,141,000 bu. of oats (1 bu. = 32 lbs.), 81% was grown in the North Central States; Iowa led with 18.4% of the total U. S. production, followed by Minnesota (13.2%), Illinois (11.4%), and Wisconsin (7.5%). The South Central States are next in importance to the North Central area; of these, Texas with 3.5% and Oklahoma with 2.6% of the U. S. production are the largest producers. White and yellow varieties are the most commonly grown in the North while red and gray oats predominate in the South.

(*e*) *Rice*

Rice is a tropical cereal and its culture is the chief agricultural industry of such densely populated rice-eating countries as China, India, Japan, and Java. It is the principal food of over half the population of the world but is of minor importance in the United States.

The cultivated rice varieties of the world fall into two general classes, the lowland and the upland. The lowland type, as the name implies, is grown on rather low, level land which is submerged under water during the major part of the growing season; the upland varieties are grown without irrigation just as corn or cotton is grown. United States commercial production is virtually confined to lowland or irrigated varieties which include long-grain, medium-grain, and short-grain (or Japanese) types, each including a number of varieties of early, medium, and late maturity.

The three principal rice-producing sections in this country are: the level prairie land of southwestern Louisiana and southeastern Texas, the Grand Prairie section of eastern Arkansas, and the Sacramento and San Joaquin valleys of California.

In Arkansas, Louisiana, and Texas, the long-grain varieties represented 26.2%, the medium-grain 71.3%, and the short-grain type only 2.3%, of the total production, while in California, the short-grain or Japanese types comprised 99.3% of all the rice grown in 1939.[5] The leading long-grain varieties are Rexoro, Nira, Fortuna, Edith, and Lady Wright; the leading medium-grain varieties are Blue Rose and Early Prolific; while Caloro is the most important short-grain or "japan" type variety. The short-grain or Japanese varieties are not as popular with U. S. consumers as some of the long-grain and medium-grain types.

---

[4] R. M. Hixon and C. F. Sprague, *Ind. Eng. Chem.*, **34**, 959 (1942).

Rice is grown in a similar manner to other small grains except that after the young plants are 6 to 8 in. tall, the land is covered with water to a depth of 1 to 2 in. which is gradually increased from 4 to 6 in. during the growing season. About two weeks before maturity, when the rice is fully headed and the panicles have begun to turn down and are ripening in the upper portions, the land is drained. In order to avoid undue loss of grain from shattering, the rice is harvested when the kernels in the lower portion of the head are in the "hard dough" stage. At this time, the kernels at the top of the panicles are fully ripe and the standing grain contains from 23 to 28% moisture. The rice is cut with a binder, shocked, cured, and threshed in the same manner as other small grains. Rice is a slow-growing crop as compared with other spring-sown cereals. In the southern States, it is normally seeded from April 1 to May 15 and the crop is harvested in late September and early October. Depending upon variety and climatic conditions, 120 to 172 days elapse from date of seeding to maturity. The threshed grain is called rough rice (or paddy, in oriental countries), the yield of which is about the same as that of the straw.

(*f*) *Rye*

Rye is not an important crop in the United States. It is widely grown in North European and in Scandinavian countries where it is largely milled into rye flour for use in breadmaking. It has been called the "grain of poverty" since it does well on poor soil and in arid areas. While both spring and winter types of rye are known, the former is only of minor importance in the United States. As rye is hardier than wheat it may be grown further north from fall seeding and the chief producing areas are North Dakota, Minnesota, and South Dakota. Unlike wheat, which is almost exclusively a self-fertilized plant, but like corn, rye is open-pollinated so that much of the rye grown is not pure as to variety.

Rye is prone to attack by the fungus *Claviceps purpurea;* the sclerotia of which are commonly known as ergot, horned, or spurred rye. The kernels are replaced by the fungus giving rise to dark, purple-colored, waxlike bodies which sometimes attain a length of 1.5 in. Ergot is a valuable drug since it contains a series of alkaloids, of which ergotoxine ($C_{35}H_{41}O_6N_5$), ergotamine ($C_{33}H_{35}O_5N_5$), and ergonovine ($C_{19}H_{23}O_2N_3$) are physiologically active, and are characterized by their vasoconstrictor action and their power to cause contractions of the uterus.[6] The sterol, ergosterol, was so named because it was first discovered in ergot. In view of its poisonous properties, ergot must be removed from rye prior to milling; it has considerable commercial value because of its use for medicinal purposes.

---

[5] J. W. Jones, U. S. Dept. Agr., *Circ.* 612 (1941).

[6] G. Barger, *Ergot and Ergotism.* Gurney and Jackson, London, 1931.

(*g*) *Sorghums*

Sorghums of four general classes, namely, sorgo or sweet sorghum, broom corn, grass sorghums, and grain sorghums, are grown in the United States. They thrive well under drought conditions and are grown principally in the southern sections of the Great Plains area and in parts of the Southwest. There are five types of grain sorghums, with a number of varieties in each, which differ in the color of the seed and hull. The sorghums are used principally for livestock feeding in this country and are at present of minor interest to the food technologist. A portion of the acreage of sweet sorghum is used for the production of sorghum sirup. A red sorghum variety, Leoti, yields waxy starch and the sorghums may become of importance as one of the sources of waxy starch to replace tapioca.

(*h*) *Wheat*

Wheat is adapted to all moderately dry temperate regions. Three botanical species, namely, *Triticum vulgare* (common wheat), *T. durum* (amber and red durum wheats), and *T. compactum* (white and red club wheat), each representing many varieties, are grown in the United States but members of the *vulgare* group represent nearly 95% of the total production. Common or *vulgare* wheats differ widely in morphological characters such as the presence or absence of awns, color (red and white), and kernel texture (hard and soft), as well as in physiological characters (such as spring or winter habit, yield, earliness, resistance to drought, cold, and disease), and in milling and baking behavior. For commercial purposes, they are classified into four major groups: hard red spring, hard red winter, soft red winter, and white wheats (winter and spring). Spring wheat is sown in the spring and harvested in the late summer whereas winter wheat is planted in the fall and develops a root system before the onset of cold weather. In the spring, it makes a vigorous growth and is harvested in the early summer. For the 10-year period, 1930 to 1939, inclusive, hard red winter wheat represented 41.7%; soft red winter 27.6%; hard red spring 14.9%, white wheats (including white club) 11.9%, and durum (amber and red) 3.9% of the average annual production of 747,507,000 bu. The principal hard red spring wheat areas are in the northern portion of the Great Plains area, in the States of Minnesota, North Dakota, South Dakota, and Montana, where semiarid conditions prevail. The hard red winter wheat region is principally in the South Central and North Central States, the greatest acreages occurring in Kansas, Nebraska, Oklahoma, and Texas. Soft red winter wheat is chiefly grown east of the Great Plains area and south of Wisconsin. It is the leading class of wheat in Ohio, Indiana, Illinois, Missouri, Pennsylvania, Maryland, Virginia, West Virginia, New Jersey, Delaware, North Carolina, South Carolina, Georgia, Kentucky, Tennessee, and Alabama. White wheat is grown principally in the Pacific Coast States and in New York and Michigan. White Club is grown only to a minor extent, chiefly in the Pacific Coast States and in the States of Utah and Idaho.

Durum wheat, like the club wheats, belongs to a different botanical species from the common, or *vulgare*, wheats and is grown in the more arid wheat-producing areas of the world such as the Mediterranean basin, southeast Russia, western Asia, British India, part of the Great Plains region of the northern United States and western Canada and in Argentina. Durum wheat was introduced into the United States from Russia during the latter part of the 19th century. As it was resistant to certain races of stem rust which attacked existing varieties of hard red spring wheat and required less rainfall, it was particularly adapted to the climatic conditions normally prevailing in the northcentral region of North Dakota, and in portions of South Dakota, Montana, and Minnesota (Red River Valley), as well as to the southern sections of the neighboring Western Canadian provinces of Manitoba, and eastern Saskatchewan.[7, 8] For the crop years of 1930–1939, inclusive, 75% of the durum wheat was produced in North Dakota and 20% in South Dakota. The difference in rust resistance between durum and hard red spring wheat varieties is becoming narrower from year to year, as a result of the introduction of new rust-resistant hard red spring wheats of high breadmaking quality, such as Thatcher (released by the Minnesota Agricultural Experiment Station in 1934 and now the leading variety of this class).

Some varieties of durum wheat yield grain of an amber color, while in others the grain is red in color. The amber durums are prized for the manufacture of macaroni products; red durums are not in demand by durum wheat millers and it is not surprising that the amber varieties are chiefly grown.

3. Kernel Structure

(*a*) *General Structure of Cereal Grains*

The grain or kernel of a cereal is really a nutlike fruit, or caryopsis. The fruit contains only one seed and, as it ripens, the ovary wall, or pericarp, becomes rather firmly attached to the wall of the seed proper and forms the outer tissue of the bran. The plantlet, or embryo, which is monocotyledonous and develops into a new plant upon germination, occupies only a small part of the seed. The bulk of the seed is taken up by the floury portion, or endosperm, which constitutes a food reservoir.

In the grass family, the floral envelopes (modified leaves), or chaffy parts, within which the caryopsis develops, persist to maturity. In some cereals such as rice, most varieties of oats and barley, certain of the chaffy structures envelop the caryopsis so closely that they remain attached to it when the grain is threshed; these structures constitute the hull of such grains (which are said to be covered). In the common wheats, rye, hull-less barleys, and the common varieties of corn, the caryopsis readily separates from the floral envelopes on threshing; these grains are said to be naked. The microscopic structure of the various cereal grains is very similar and will only be exemplified with a few grains.

(*b*) *Wheat*

As shown in Fig. 51, one side of the wheat grain, known as the dorsal side, is rounded while the other side (the ventral side) has a deep groove or crease (not shown in the

---

[7] C. L. Alsberg, *Wheat Studies, Food Research Inst.*, **15**, 337 (1939).

[8] J. A. Clark and K. S. Quisenberry, U. S. Dept. Agr., *Circ.* **634** (1942).

longitudinal section) which extends the entire length of the kernel. At the apex or small end (stigmatic end) of the grain there is a cluster of short fine hairs known as brush hairs. The pericarp, or dry fruit coat, which corresponds to the shell of a nut, consists of three outer bran layers (epidermis, epicarp, and endocarp). The remaining tissues comprise the seed proper and consist of the inner bran, endosperm, and embryo (germ). The aleurone layer, sometimes erroneously called the gluten layer, consists of large rectangular, heavy-walled, starch-free cells; botanically it is the outer layer of the endosperm, but as it tends to remain attached to the outer coats during the milling process, it is shown in the diagram as the innermost bran layer

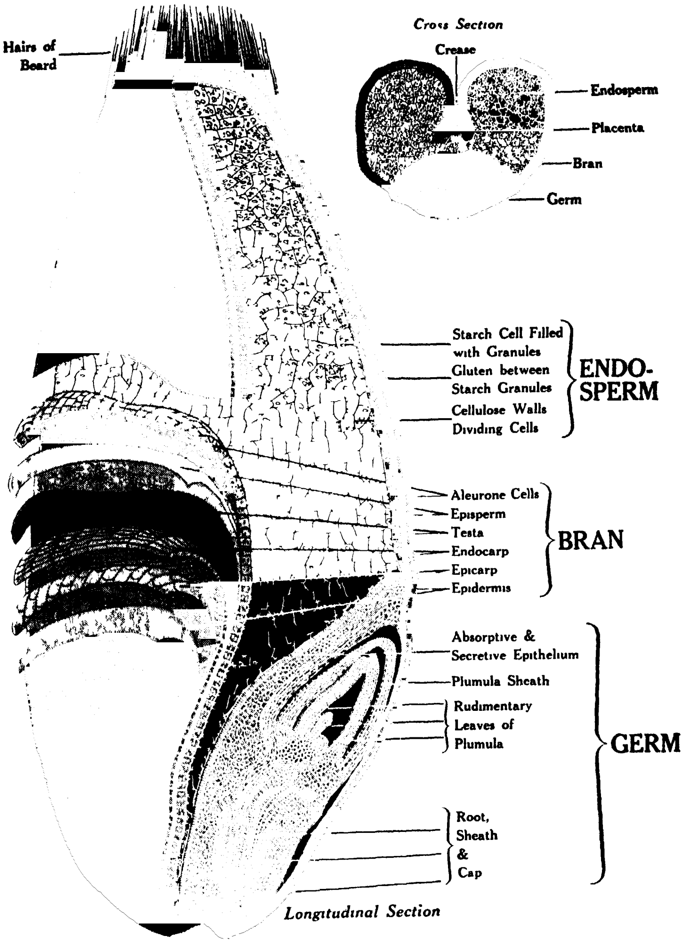


Fig 51 —Structure of the wheat kernel.

The embryo (germ), or plantlet, consists of the plumule and radicle which are connected by the hypocotyl; this, in turn, is connected with the scutellum which serves as a special organ for absorbing the food products from the endosperm and transferring them to the plantlet. The surface of the scutellum which adjoins the endosperm consists of a layer of columnar cells known as the epidermal layer or epithelial layer of the scutellum. These epithelial cells contain prominent nuclei and assume a glandular, or enzyme-secreting, function during germination of the seed. In a well-filled wheat kernel, the germ comprises about 2 to 3% of the kernel, the bran 13 to 17%, and the endosperm the remainder. The bran layers are high in protein, cellulose, hemicelluloses, and mineral constituents; biologically, they function as a protective coating and remain practically intact when the seed germinates. The germ is rich in proteins, lipids, sugar (chiefly sucrose), and ash constituents, while the endosperm consists largely of starch grains embedded in a matrix of protein.

(*c*) *Buckwheat*

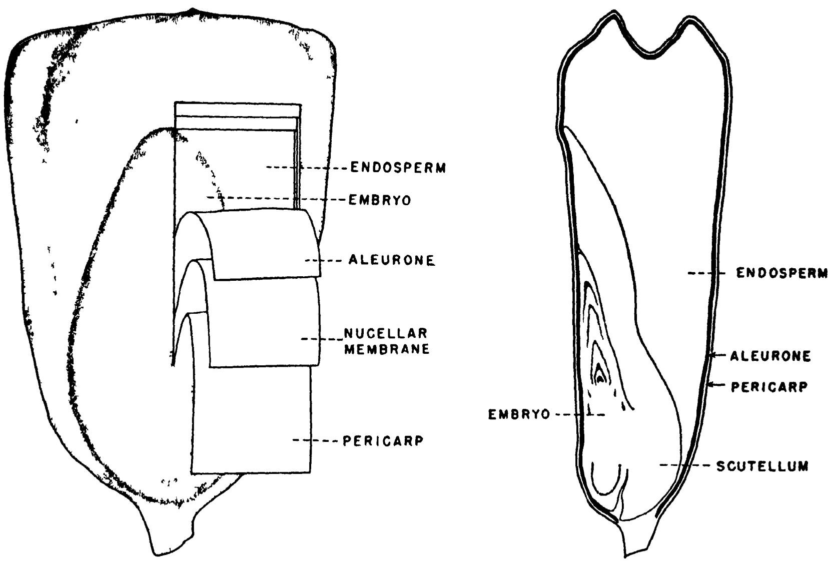
In the buckwheat grain, the floral envelopes are absent. The kernels, or achenes, are triangular in shape, dull gray or dark brown in color, with a thick fibrous pericarp, which is not fused or cemented to the seed as in the true cereals. In common with the grains of the grass family, buckwheat has an aleurone layer of large starch-free cells next to the endosperm. The embryo is dicotyledonous and is embedded in the white endosperm which forms the bulk of the seed. It is, however, largely removed in the milling process.

(*d*) *Corn*

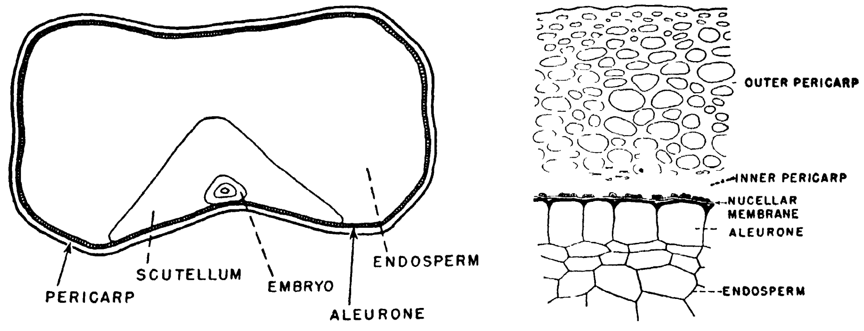
The mature grain of corn is larger than that of any other cereals but the different types vary considerably in size, shape, and hardness. In dent corn, as shown in Fig. 52, the kernel is flattened, wedge-shaped, and is broader at the apex than at the point of attachment to the cob. Flint varieties have a somewhat more rounded top. The kernels of sweet corn are translucent, horny and wrinkled in appearance, the starch being partially replaced by sugar. Popcorn is characterized by a hard corneous endosperm and small kernels of two main types: rice, with pointed kernels, and pearl, with rounded kernels. Popcorn has the ability to explode or pop because of the pressure exerted by the water vapor produced upon the application of heat.

The aleurone cells contain the pigments which give rise to the blue, black, or purple color of certain varieties; this outer layer consists largely of "horny gluten." Inside of this, "horny starch" and "white, or floury, starch" are found. In dent varieties, the horny starch, consisting essentially of a mixture of starch and gluten, is found on the sides and back of the kernel and bulges in toward the center while the white or floury starch fills the crown or upper part of the kernel and extends downward to surround the germ. In the maturation of dent corn, the soft floury starch at the top of the grain shrinks and this is responsible for the indentation at the top of the kernels. In flint varieties of corn, the horny starch extends around the top of the kernel so that the floury portion of the endosperm is completely enclosed by a very hard outer layer.

In dent corn, the hull comprises about 6%, the germ 11%, and the endosperm 83% of the kernel.



WHOLE GRAIN LONGITUDINAL SECTION



CROSS SECTION LAYERS OF THE KERNEL OF CORN

Fig. 52.—Structure of the corn kernel (Dent variety) (Prepared by U S Dept Agr., Washington, D. C)

(e) Oats

In the common varieties of oats, the fruit or caryopsis is enveloped in a hull comprising certain of the floral envelopes; naked or hull-less varieties exist but they are not extensively grown.

The percentage hull in oats varies with the weight per measured bushel. In light, thin oats of low test weight, the hulls may comprise as much as 45% of the grain, while in very heavy or plump oats, they may represent only 20% of the oat. While test weights of oats vary from about 20 to 50 lbs., they average from 32 to 36 lbs. and the hull normally makes up about 30% of the grain. Kernels of oats, obtained by removing the hulls, are called groats. A drawing of an oat kernel or groat is reproduced in Fig. 53; in general structure, it is similar to the caryopsis of other cereal grains, but has a fuzzy covering of trichomes or hairs.

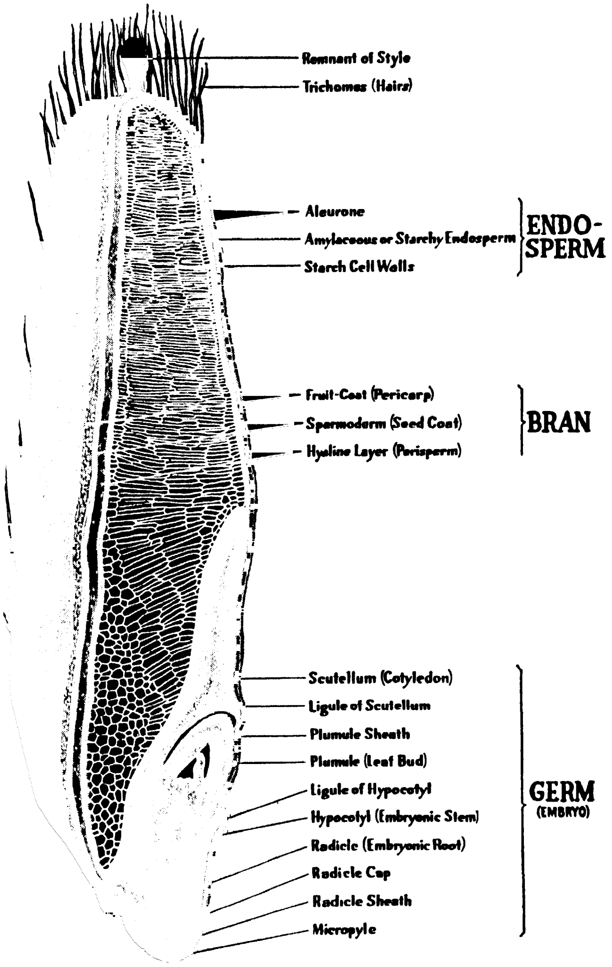


Fig. 53 Kernel, or groat, of oats from which the hull has been removed. (By courtesy of The Quaker Oats Milling Co. Drawing by Andrew L. Winton, Ph.D.)

4. Commercial Classification of Grain. U. S. Grain Standards

In order that grain might be marketed economically and advantageously under uniform standards, the United States Grain Standards Act was passed in 1916. This Act provided for the establishment of official grain standards for the common grains and the federal licensing and supervision of the work of the grain inspectors.[9] The

TABLE 113

CLASSES AND SUBCLASSES OF WHEAT PROVIDED BY THE FEDERAL GRAIN STANDARDS

Class	Subclass	Chief requirements
I. Hard Red Spring Wheat	A. Dark Northern Spring	75% or more of dark, hard, vitreous kernels; not more than 10% of the variety Humpback
	B. Northern Spring	25 to 75% of dark, hard, vitreous kernels; not more than 10% of the variety Humpback
	C. Red Spring	Less than 25% of dark, hard, vitreous kernels or Hard Red Spring Wheat consisting of more than 10% of the variety Humpback
II. Durum Wheat	A. Hard Amber Durum	75% or more of hard, vitreous, amber kernels
	B. Amber Durum	60–75% of hard, vitreous, amber kernels
	C. Durum	Less than 60% of hard, vitreous, amber kernels
III. Red Durum Wheat	....	All varieties of Red Durum
IV. Hard Red Winter Wheat	A. Dark Hard Winter	75% or more of dark, hard, vitreous kernels
	B. Hard Winter	25–75% of dark, hard, vitreous kernels
	C. Yellow Hard Winter	Less than 25% of dark, hard, vitreous kernels
V. Soft Red Winter Wheat	A. Red Winter	Shall contain not more than 10% of Soft Red Winter Wheat grown west of the Great Plains area
	B. Western Red	Shall contain more than 10% of Soft Red Winter Wheat grown west of the Great Plains area
VI. White Wheat	A. Hard White	75% or more of hard kernels
	B. Soft White	Less than 75% of hard kernels and not more than 10% of Sonora and/or White Club varieties
	C. White Club	Not more than 10% of common white wheat other than Sonora or White Club varieties
	D. Western White	Contains more than 10% of Sonora wheat and/or White Club varieties and also contains more than 10% of common white wheat other than Sonora
VII. Mixed Wheat[a]	....	....

[a] Classes I to VI, inclusive, must not include more than 10% of wheats of other classes. Class VII includes all mixtures not provided for in Classes I to VI; in grading this class the grade designation shows the name and approximate percentage of each class of wheat which constitutes more than 10% of the mixture. Special mixed grades are also provided for "amber mixed durum" and "mixed durum."

---

[9] W. P. Carroll and A. B. Combs, U. S. Dept. Agr., *Misc. Pub.* 325 (1938).

standards have been revised and extended from time to time and those at present in force for all the cereal grains except rice are published in the *Handbook of Official Grain Standards of the United States.*[10] The standards are designed to provide market classifications for grains which differ in quality as a result of type, variety, and production conditions, by sortation into classes, and frequently subclasses, with a number of grades in each.

(*a*) *Wheat*

As shown in Table 113, wheat is divided into seven classes on the basis of wheat type and color; certain of the classes are further divided into subclasses chiefly on the basis of kernel density or geographical origin of the wheat; although in a few cases the presence of certain varieties, which differ in inherent quality from other members of the group, is taken into consideration. The intent is to effect a separation which takes cognizance of the suitability of the wheat for specific purposes. Hard red spring, hard red winter, and hard white wheats are valued for the production of flours used in making high-quality yeast-leavened bread; soft red winter, soft white, and white club

TABLE 114

U. S. GRAIN STANDARDS FOR WHEAT

CLASS I—HARD RED SPRING WHEAT

Grade requirements for (a) Dark Northern Spring, (b) Northern Spring, (c) Red Spring

Grade No.	Minimum test wt. per bu., lbs.	Maximum limits of—					
		Damaged kernels (wheat and other grains)		Foreign material		Wheats of other classes	
		Total, %	Heat-damaged, %	Total, %	Matter except other grains, %	Total, %	Durum and/or Red Durum, %
1 Heavy[a, b]	60	2	0.1	1	0.5	5	2
1[b]	58	2	0.1	1	0.5	5	2
2[b]	57	4	0.2	2	1.0	10	3
3	55	7	0.5	3	2.0	10	5
4	53	10	1.0	5	3.0	10	10
5	50	15	3.0	7	5.0	10	10
Sample grade	Sample grade shall include wheat of the subclass Dark Northern Spring, or Northern Spring, or Red Spring, which does not come within the requirements of any of the grades from No. 1 heavy to No. 5, inclusive; or which contains more than 16% of moisture; or which contains inseparable stones and/or cinders; or which is musty, or sour, or heating, or hot; or which has any commercially objectionable foreign odor except of smut or garlic; or which contains a quantity of smut so great that any one or more of the grade requirements cannot be applied accurately; or which is otherwise of distinctly low quality.						

[a] Applies to each of the subclasses Dark Northern Spring, Northern Spring, and Red Spring.

[b] The wheat in grades No. 1 Heavy and No. 1 of this class may contain not more than 7%, and the wheat in grade No. 2 of this class may contain not more than 10%, of shrunken and/or broken kernels of grain and other matter that will pass through a 20-gage metal sieve with slotted perforations 0.064 in. wide by $^3/_8$ in. long.

---

[10] U S. Dept. Agr., Agricultural Marketing Service, *U. S. G. S. A. Form* 90 (revised 1941).

CLASS II—DURUM WHEAT AND CLASS III—RED DURUM WHEAT

Grade requirements for the subclasses (a) Hard Amber Durum, (b) Amber Durum, and (c) Durum, of the class Durum Wheat, and for the class Red Durum Wheat

Grade No.	Minimum test wt. per bu., lbs.	Maximum limits of—					
		Damaged kernels (wheat and other grains)		Foreign material		Wheats of other classes	
		Total, %	Heat-damaged, %	Total, %	Matter except other grains, %	Total, %	Soft Red Winter, White, and Red Durum, singly or combined,[a] %
1[c]............	60	2	0.1	1	0.5	5[b]	3
2[c]............	58	4	0.2	2	1.0	10	5
3[c]............	56	7	0.5	3	2.0	10	10
4.............	54	10	1.0	5	3.0	10	10
5.............	51	15	3.0	7	5.0	10	10
Sample grade..	Sample grade shall include wheat of the subclass Hard Amber Durum, or Amber Durum, or Durum, or wheat of the class Red Durum Wheat, which does not come within the requirements of any of the grades from No. 1 to No. 5, inclusive; or which contains more than 16% of moisture; or which contains inseparable stones and/or cinders; or which is musty, or sour, or heating, or hot; or which has any commercially objectionable foreign odor except of smut or garlic; or which contains a quantity of smut so great that any one or more of the grade requirements cannot be applied accurately; or which is otherwise of distinctly low quality.						

---

[a] These specifications do not apply to the class Red Durum Wheat, or to the subclass Durum.

[b] No. 1 red durum may contain 10% of wheats of other classes.

[c] The wheat in grades No. 1 and No. 2 of each of these classes may contain not more than either (*a*) 7% of shrunken and/or broken kernels of grain and other matter that will pass through a 20-gage metal sieve with slotted perforations 0.064 in. wide by $^3/_8$ in. long or (*b*) 10% of all such material that will pass through said sieve together with the broken kernels of grain of any size which remain on said sieve; and the wheat in grade No. 3 of each of these classes may contain not more than either (*a*) 10% of shrunken and/or broken kernels of grain and other matter that will pass through said sieve, or (*b*) 15% of all such material that will pass through said sieve together with the broken kernels of grain of any size which remain on said sieve.

CLASS IV—HARD RED WINTER WHEAT

Grade requirements for (a) Dark Hard Winter, (b) Hard Winter, (c) Yellow Hard Winter

Grade No.	Minimum test wt. per bu., lbs.	Maximum limits of—					
		Damaged kernels (wheat and other grains)		Foreign material		Wheats of other classes	
		Total, %	Heat-damaged, %	Total, %	Matter except other grains, %	Total, %	Durum and/or Red Durum, %
1[a]............	60	2	0.1	1	0.5	5	1
2[a]............	58	4	0.2	2	1.0	10	2
3[a]............	56	7	0.5	3	2.0	10	3
4.............	54	10	1.0	5	3.0	10	10
5.............	51	15	3.0	7	5.0	10	10
Sample grade..	Sample grade shall include wheat of the subclass Dark Hard Winter, or Hard Winter, or Yellow Hard Winter, which does not come within the requirements of any of the grades from No. 1 to No. 5, inclusive; or which contains more than 15.5% of moisture; or which contains inseparable stones and/or cinders; or which is musty, or sour, or heating, or hot; or which has any commercially objectionable foreign odor except of smut or garlic; or which contains a quantity of smut so great that any one or more of the grade requirements cannot be applied accurately; or which is otherwise of distinctly low quality.						

---

[a] The wheat in grades No. 1 and No. 2 of this class may contain not more than 7%, and the wheat in grade No. 3 of this class may contain not more than 10%, of shrunken and/or broken kernels of grain and other matter that will pass through a 20-gage metal sieve with slotted perforations 0.064 in. wide by $^3/_8$ in. long.

Class V—Soft Red Winter Wheat

Grade requirements for (a) Red Winter, (b) Western Red

Grade No.	Minimum test wt. per bu., lbs.	Maximum limits of—					
		Damaged kernels (wheat and other grains)		Foreign material		Wheats of other classes	
		Total, %	Heat-damaged, %	Total, %	Matter except other grains, %	Total, %	Durum and/or Red Durum, %
1[a]	60	2	0.1	1	0.5	5	0.5
2[a]	58	4	0.2	2	1.0	10	1.0
3[a]	56	7	0.5	3	2.0	10	2.0
4	54	10	1.0	5	3.0	10	10.0
5	51	15	3.0	7	5.0	10	10.0
Sample grade	Sample grade shall include wheat of the subclass Red Winter, or Western Red, which does not come within the requirements of any of the grades from No. 1 to No. 5 inclusive; or which contains more than 15.5% of moisture; or which contains inseparable stones and/or cinders; or which is musty, or sour, or heating, or hot; or which has any commercially objectionable foreign odor except of smut or garlic; or which contains a quantity of smut so great that any one or more of the grade requirements cannot be applied accurately; or which is otherwise of distinctly low quality.						

[a] The wheat in grades No. 1 and No. 2 of this class may contain not more than 7%, and the wheat in grade No. 3 of this class may contain not more than 10%, of shrunken and/or broken kernels of grain and other matter that will pass through a 20-gage metal sieve with slotted perforations 0.064 in. wide by $^3/_8$ in. long.

Class VI—White Wheat

Grade requirements for (a) Hard White, (b) Soft White, (c) White Club, (d) Western White

Grade No.	Minimum test wt. per bu., lbs.	Maximum limits of—					
		Damaged kernels (wheat and other grains)		Foreign material		Wheats of other classes	
		Total, %	Heat-damaged, %	Total, %	Matter except other grains, %	Total, %	Durum and/or Red Durum, %
1[a]	60	2	0.1	1	0.5	5	0.5
2[a]	58	4	0.2	2	1.0	10	1.0
3[a]	56	7	0.5	3	2.0	10	2.0
4	54	10	1.0	5	3.0	10	10.0
5	51	15	3.0	7	5.0	10	10.0
Sample grade	Sample grade shall include wheat of the subclass Hard White, or Soft White, or White Club, or Western White, which does not come within the requirements of any of the grades from No. 1 to No. 5, inclusive; or which contains more than 15.5% of moisture; or which contains inseparable stones and/or cinders; or which is musty, or sour, or heating, or hot; or which has any commercially objectionable foreign odor except of smut or garlic; or which contains a quantity of smut so great that any one or more of the grade requirements cannot be applied accurately; or which is otherwise of distinctly low quality.						

[a] The wheat in grades No. 1 and No. 2 of this class may contain not more than 7%, and the wheat in grade No. 3 of this class may contain not more than 10%, of shrunken and/or broken kernels of grain and other matter that will pass through a 20-gage metal sieve with slotted perforations 0.064 in. wide by $^3/_8$ in. long.

wheats are especially suited for making chemically leavened baked products; amber durum wheat is prized for the manufacture of semolina and flour used in the manufacture of alimentary pastes (macaroni, vermicelli, etc.); while red durum is used principally as a poultry and livestock feed. The protein content of wheat is of great importance in relation to its suitability for these purposes. Kernel texture is a useful factor in making the classifications since the vitreousness or hardness of the kernels is positively associated in a general way with protein content.

The grain in each subclass is sorted into a number of grades on the basis of "quality" and "condition" characteristics. In general, quality refers to the plumpness, soundness, and cleanliness of the grain; these properties are measured by determining the test-weight per bushel, "damaged kernels" or "sound kernels," and "dockage" or "foreign material." The condition refers to the moisture content, odor, presence of smut, garlic, insects, and to the extent of weathering or artificial treatment to which the grain has been subjected. The grade requirements for the various classes of subclasses of wheat are reproduced in Table 114. It will be seen that the "quality" requirements become less stringent with a decrease in the grade. Test weight per bushel is an index of the endosperm content of the grain and hence of potential flour yield. Moisture content is important in relation to the keeping quality of the grain in storage as well as in regard to the yield of the various manufactured products prepared from it. For wheat (also rye, barley, and oats) the standards specify maximum moisture limits for all the regular grades and when the moisture exceeds this limit but is below a certain higher maximum, the word "tough" is added to the grade designation. For the hard red spring, durum wheat, and mixed wheat classes, the designation "Tough" is added when the moisture content falls between 14.5 and 16.0%; for the remaining classes the moisture limits are 14.0 to 15.5%. Special grades are also provided for wheat which contains garlic, smutty grain, excess moisture, etc., but which would otherwise be eligible for one of the regular grades.

The same general principles are applied in the grading of the other grains.

(*b*) *Barley*

Barley is divided into four classes on the basis of color and areas of production, namely: Class I, barley; Class II, black barley: Class III, western barley; and Class

TABLE 115

U. S. GRAIN STANDARDS FOR BARLEY

BARLEY (CLASS I)

This class shall include all white (glumes) barley grown east of the Rocky Mountains, and may include not more than 10% of barley of other classes. This class shall be divided into two subclasses, as follows:

Subclass (A) Malting Barley

This subclass shall include 6-rowed barley of the class Barley (Class I) which meets the requirements of grades Nos. 1 to 3, inclusive, which, after the removal of dockage, contains not more than 5% of 2-rowed and/or other types or varieties of barley of unsuitable malting type such as Trebi and Black; which contains not more than 15% of barley and other matter that will pass through a 20-gage metal sieve with slotted perforations 0.076 ($4^7/_8/64$) of an inch wide and $^3/_4$ of an inch long; which contains not more than 5% of skinned and/or broken kernels; which contains not more than 4% of damaged barley; and shall not include bleached barley. Barley of this subclass shall contain 75% or more of mellow barley kernels which kernels are not, en masse, semi-steely.

Subclass (B) Barley

This subclass shall include all barley of the class Barley which does not meet the requirements of subclass (A) Malting Barley.

BLACK BARLEY (CLASS II)

This class shall include all varieties of black (glumes) barley grown anywhere in the United States, and may include not more than 10% of barley of other classes.

WESTERN BARLEY (CLASS III)

This class shall include white (glumes) barley grown west of the Great Plains area of the United States, and may include not more than 10% of barley of other classes.

MIXED BARLEY (CLASS IV)

This class shall be any mixture of barley not provided for in the classes from I to III, inclusive.

Grade requirements and designations.—Mixed Barley shall be graded according to the grade requirements of either (1) the subclass Barley of the class Barley, or (2) the class Black Barley, or (3) the class Western Barley, according to which class of barley predominates in the mixture, except that all grade specifications as to the maximum percentages of black barley shall be disregarded.

The grade designation for Mixed Barley shall include successively, in the order named, the number of the grade or the words "Sample Grade," as the case may be; the words "Mixed Barley," followed by the name and approximate percentage of each class of barley which constitutes 10% or more of the mixture in the order of its predominance, but if only one class exceeds 10% of the mixture, the name and approximate percentage of that class shall be included in the grade designation, followed by the name and approximate percentage of at least one other class. For Mixed Barley, in which barley of the class Western Barley predominates, the grade designation shall include a statement of the test weight per bushel immediately following the names and percentages of the classes composing the mixture.

CLASS I—BARLEY AND CLASS II—BLACK BARLEY

Grade requirements for subclass (a) Malting Barley and subclass (b) Barley, of the class Barley, and for the class Black Barley

Grade No.	Minimum limits of—		Maximum limits of—			
	Test wt. per bu., lbs.	Sound barley,[a] %	Heat-damaged kernels (barley, other grains, and wild oats), %	Foreign material, %	Broken kernels, %	Black barley,[b] %
1[c]	47	95	0.1	1	4	0.5
2[c]	46	93	0.2	2	8	1.0
3[c]	43	90	0.5	3	12	2.0
4[d]	40	80	1.0	4	20	5.0
5	35	70	3.0	6	30	10.0
Sample grade	Sample grade shall include barley of the subclass Barley, or of the class Black Barley, which does not come within the grade requirements of any of the grades from No. 1 to No. 5, inclusive; or which contains more than 16% of moisture; or which contains inseparable stones and/or cinders; or which is musty, or sour, or heating, or hot; or which has any commercially objectionable foreign odor except of smut or garlic; or which contains a quantity of smut so great that any one or more of the grade requirements cannot be applied accurately; or which is otherwise of distinctly low quality.					

---

[a] Any barley in grade No. 1 that does not come within the provisions of the special grade Blighted, may contain not more than 2% of blight-damaged barley; and barley in any grade from No. 2 to Sample grade, inclusive, that does not come within the provisions of the special grade Blighted, may contain not more than 4% of blight-damaged barley. Any barley containing more than 4% of blight-damaged barley shall be graded No. 1, No. 2, No. 3, No. 4, No. 5, or Sample grade, Blighted, as the case may be, as provided in the specifications for Blighted Barley.

[b] These specifications do not apply to the class Black Barley.

[c] See special requirements for subclass (A) Malting Barley.

[d] Barley, that is badly stained or materially weathered, shall not be graded higher than No. 4.

CLASS III—WESTERN BARLEY

Grade requirements for Western Barley

Grade No.	Minimum limits of sound barley, %	Maximum limits of—				
		Heat-damaged kernels (barley, other grains, and wild oats), %	Wild oats, %	Foreign material, %	Broken kernels, %	Black barley, %
1	98	0.1	1	0.5	3	0.5
2	96	0.2	2	1.0	6	1.0
3	93	0.3	3	2.0	10	2.0
4	88	0.5	5	3.0	15	5.0
5	80	1.0	10	4.0	25	10.0
Sample grade	Sample grade shall include barley of the class Western Barley which does not come within the grade requirements of any of the grades from No. 1 to No. 5, inclusive; or which contains more than 15% of moisture; or which contains inseparable adobe, stones, and cinders, singly or combined; or which is musty, or sour, or heating, or hot; or which has any commercially objectionable foreign odor except of smut or garlic; or which contains a quantity of smut so great that any one or more of the grade requirements cannot be applied accurately; or which contains the seeds of wild brome grasses of a character and in a quantity sufficient to cause the grain to be of low quality for feeding purposes; or which is otherwise of distinctly low quality.					

IV, mixed barley. Black barley (black glumes) is thus placed in a separate class from white barley. Class I barley comprises all white (glumes) barley grown east of the Rocky Mountains; it may include not more than 10% of barley of other classes. A special subclass, malting barley, is provided for six-row barley which is particularly suitable for malting purposes. The standards for barley are given in Table 115. Western barley (or mixed barley, in which western barley predominates), which contains more than 13.5% but not more than 15% moisture, has the word "Tough" added to the grade. Other barleys are graded "Tough" when the moisture content exceeds 14.5% but not more than 16%. Special grades are provided for bright western barley, stained western barley, and for blighted, smutted, garlicky, and weevily barley.

(*c*) *Corn*

Corn is divided into three classes, namely: Class I, yellow corn; Class II, white corn; and Class III, mixed corn. These class designations apply when the corn is of the dent type and the word "Flint" is added when the corn consists of 95% or more of flint varieties. When the corn consists of more than 5 but less than 95% flint varieties, the designation "flint and dent" is added to the above class names. In the instance of corn (and also the grain sorghums) maximum moisture limits are included in the grade specifications. These cereals vary considerably in moisture content as they come upon the market and the use of moisture as a direct grading factor is essential to indicate their dry-matter values and their storage properties; in fact, moisture content is the principal factor determining the grade of market corn. The grade requirements for corn are given in Table 116.

TABLE 116

U. S. GRAIN STANDARDS FOR CORN

Grade Requirements for Yellow Corn, White Corn, and Mixed Corn

Grade No.	Minimum test wt. per bu., lbs.	Maximum limits of—			
		Moisture, %	Cracked corn and foreign material, %	Damaged kernels, %	
				Total	Heat-damaged
1	54	14.0	2	3	0.1
2	53	15.5	3	5	0.2
3	51	17.5	4	7	0.5
4	48	20.0	5	10	1.0
5	44	23.0	7	15	3.0
Sample grade	Sample grade shall include corn of the class Yellow Corn, or White Corn, or Mixed Corn, which does not come within the requirements of any of the grades from No. 1 to No. 5, inclusive; or which contains stones and/or cinders; or which is musty, or sour, or heating, or hot; or which has any commercially objectionable foreign odor; or which is otherwise of distinctly low quality.				

(*d*) *Oats*

Oats are divided into classes on the basis of color, as follows: Class I, white oats (includes yellow oats); Class II, red oats; Class III, gray oats; Class IV, black oats; and Class V, mixed oats. The numerical grade requirements are given in Table 117. Oats containing more than 14.5% but not more than 16% moisture have the word "Tough" added to the grade designation. Special grades are provided for heavy, extra heavy, bright, thin, bleached, weevily, smutty, ergoty, and garlicky oats.

(*e*) *Rice*

Rough rice is divided into eleven classes on the basis of variety with six numerical and one sample grade in each class: The principal factors in establishing the grade are: total damaged and heat-damaged kernels, red rice, chalky kernels, foreign material (cereal grains, weed seeds), and rice of other classes. In the majority of the classes, provision is made for the determination of milling quality based on the use of a shelling device;[11] the milling quality is stated in hyphenated form as part of the grade designation of all rough rice to which it applies. There are many special grades of rough rice.

(*f*) *Rye*

The grade requirements for rye are given in Table 118. The designation "Tough" is added when sample contains more than 14% but not more than 16% moisture. Special grades are also provided for smutty, garlicky, weevily, and ergoty rye. The word "ergoty" is made a part of the grade designation when the rye contains more than 0.3% ergot.

---

[11] U. S. Dept. Agr., *Circ.* **48** (Oct., 1928).

Table 117

U. S. Grain Standards for Oats

Grade Requirements for the Classes White Oats, Red Oats, Black Oats, and Mixed Oats

---

Standards[a]

For the purposes of the official grain standards of the United States for oats:

Definition.—Oats shall be any grain which consists of 80% or more of cultivated oats. Oats may contain not more than 10% of wild oats.

Classes.—Oats shall be divided into five classes as follows: Class I, White Oats; Class II, Red Oats; Class III, Gray Oats; Class IV, Black Oats; and Class V, Mixed Oats. For the purpose of this classification, the characteristics of each class, except Mixed Oats, shall be based on color characteristics; White Oats shall include yellow oats; and tinges of white, brown, or black, on the kernels of any red oats variety shall not affect their classification as red oats. Oats of any class except Mixed Oats may include not more than 10% of cultivated oats of other classes. Mixed Oats shall be any mixture of oats which does not meet the requirements for any one of the classes White Oats, Red Oats, Gray Oats, or Black Oats.

Grades.—Oats shall be graded and designated according to the respective grade requirements of the numerical grades and sample grade of their appropriate class, and according to the special grades when applicable.

Grade requirements

Grade No.	Minimum limits of		Maximum limits of—		
	Test wt. per bu., lb.	Sound cultivated oats, %	Heat-damaged kernels (oats, other grains, and wild oats), %	Foreign material, %	Wild oats, %
1[b]	32	97	0.1	2	2
2[c]	30	94	0.3	3	3
3[d]	27	90	1.0	4	5
4[e]	24	80	3.0	5	10
Sample grade	Sample grade shall include oats of any one of the classes White Oats, Red Oats, Gray Oats, Black Oats, or Mixed Oats, which do not come within the requirements of any of the grades from No. 1 to No. 4, inclusive; or which contain more than 16% of moisture; or which contain stones and/or cinders; or which are musty, or sour, or heating, or hot; or which have any commercially objectionable foreign odor except of smut or garlic; or which contain seeds of wild brome grasses of a character and in a quantity sufficient to cause the grain to be of low quality for feeding purposes; or which are otherwise of distinctly low quality.				

---

[a] The specifications of these standards shall not excuse failure to comply with the provisions of the Federal Food, Drug, and Cosmetic Act.

[b] The oats in grade No. 1 White Oats may contain not more than 5% of oats of other classes, of which not more than 3% may be black cultivated oats.

[c] The oats in grade No. 2 White Oats may contain not more than 5% of black cultivated oats.

[d] Oats that are slightly weathered shall not be graded higher than No. 3.

[e] Oats that are badly stained or materially weathered shall not be graded higher than No. 4.

TABLE 118

U. S. GRAIN STANDARDS FOR RYE

Standards[a]

For the purposes of the official grain standards of the United States for rye:

Definition.—Rye shall be any grain which, before the removal of dockage, consists of 50% or more of rye and not more than 10% of other grains for which standards have been established under the provisions of the United States Grain Standards Act.

Grades.—Rye shall be graded and designated according to the respective grade requirements of the numerical grades and sample grade of these standards, and according to the special grades when applicable.

Dockage includes weed seeds, weed stems, chaff, straw, grain other than rye, sand, dirt, and any other foreign material, which can be removed readily from the rye by the use of appropriate sieves and cleaning devices; also undeveloped, shriveled, and small pieces of rye kernels which are removed in properly separating the foreign material, and which cannot be recovered by properly rescreening or recleaning.

The quantity of dockage shall be calculated in terms of percentage based on the total weight of the grain including the dockage. The percentage of dockage so calculated, when equal to 1% or more, shall be stated in terms of whole per cent, and when less than 1% shall not be stated. A fraction of a per cent shall be disregarded. The word "dockage," together with the percentage thereof, shall be added to the grade designation.

Grade requirements

Grade No.	Minimum test wt. per bu., lbs.	Maximum limits of—			
		Damaged kernels (rye and other grains), %		Foreign material, %	
		Total	Heat-damaged	Total	Foreign matter other than wheat
1	56	2	0.1	3	1
2	54	4	0.2	6	2
3	52	7	0.5	10	4
4	49	15	3.0	10	6
Sample grade	Sample grade shall include rye which does not come within the requirements of any of the grades from No. 1 to No. 4, inclusive; or which contains more than 16% of moisture; or which contains inseparable stones and/or cinders; or which is musty, or sour, or heating, or hot; or which has any commercially objectionable foreign odor except of smut or garlic; or which contains a quantity of smut so great that any one or more of the grade requirements cannot be applied accurately; or which is otherwise of distinctly low quality.				

---

[a] The specifications of these standards shall not excuse failure to comply with the provisions of the Federal Food, Drug, and Cosmetic Act.

5. Storage Properties of the Cereal Grains

The preservation of large quantities of grain during transportation and storage frequently occasions difficulties because of heating and spoilage. Researches have shown that the heat is produced essentially from carbohydrates as a result of a succession of reactions known collectively as respiration. In normal or aerobic respiration, oxygen of the atmosphere is involved in a process of oxidation in which carbon dioxide and water are the characteristic end products. Where hexose sugars are the food materials being utilized, the process may be summed up by the equation

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 677.2 \text{ K cal.}$$

As the quantity of carbon dioxide respired is related to the heat production, its measurement serves as a convenient laboratory method for investigating the factors involved in the heating and spoilage of grain. Respiration can also take place in the absence of atmospheric oxygen; this is known as anaerobic respiration and results in the production of carbon dioxide and a number of partially oxidized and reduced end products. The thermal conductivity of grain is rather low and when respiration occurs at a sufficiently rapid rate to produce heat more quickly than it is dissipated, the temperature of the grain rises. The rise in temperature increases the rate of the respiratory processes so that a continually self-accelerating process occurs until temperatures are attained at which the respiratory enzymes are inactivated. In experiments with wheat, Bailey and Gurjar[12] observed maximum respiratory activity at 55° C., but inactivation was not complete even at 75° C., at which temperature some roasting or "bin burning" of the grain had occurred. Spontaneous heating is not only due to the respiration of the grain itself; the respiration of bacteria, molds, and insects associated with the grain frequently accounts for a large share of the respiratory activity observed.

The rate of respiration and hence the tendency of the grain to undergo spontaneous heating depend upon a number of factors including the moisture content of the grain, the temperature, the oxygen supply, the inherent characteristics and condition of the grain, length of storage, and the biological factors (bacteria, molds, and insects) involved in respiration. Moisture content is a major factor in determining the storage behavior of grain. With the cereal grains, the increase in respiratory rate with increasing moisture content is very gradual until moisture contents of from 14 to 15% are reached above which marked acceleration in the rate occurs. This is shown in Fig. 54 taken from studies reported by Bailey;[13] at equivalent moisture contents, corn had the highest, and rye the lowest, respiratory rate of the various cereal grains studied. Bailey[14] and Bailey and Gurjar[12] have shown that the length of time the grain is stored at a high moisture content influences the rate of respiration; soft wheats respire more rapidly than hard wheats and the presence of cracked, shriveled, immature, sprouted, frosted, or heat-damaged kernels increases the rate of respiration at equivalent moistures. An increase in moisture content provides more favorable conditions for the proliferation of microorganisms and the development of insects. Lindgren[15] has shown that the respiration of insects is many times that of the grain; once they became active the increase in temperature and the moisture produced by respiration render their surroundings progressively more favorable as their population increases.

As respiration proceeds in stored grain, there is a decrease in the oxygen content and an increase in the percentage of carbon dioxide in the interseed atmosphere which naturally tends to suppress the rate of aerobic respiration.

It has long been known that deterioration of grain in storage is accompanied by an increase in acidity. Coleman and Zeleny[16] and Zeleny[17] have shown that fat acidity in particular is a useful index of the soundness or condition of wheat and corn and of their tendency to heat upon further storage. Grain may become "sour" and unfit for human consumption with little or no actual heating when the storage is prolonged. This seems to be associated with the accumulation of carbon dioxide which appears to favor the production of acid substances which interfere with the normal respiratory processes. Wheat which has suffered this type of damage is known as "sick wheat"—the germ darkens and becomes rancid, indicative of deep-seated changes.

---

[12] C. H. Bailey and A. M. Gurjar, *J. Agr. Research,* **12,** 685 (1918).

[13] C. H. Bailey, *Plant Physiol.,* **15,** 257 (1940).

[14] C. H. Bailey, Minn. Agr. Expt. Sta., *Tech. Bull.* **3** (1921).

[15] D. L. Lindgren, Minn. Agr. Expt. Sta., *Tech. Bull.* **109** (1935).

[16] D. A. Coleman and L. Zeleny, *Cereal Chem.,* **15,** 580 (1938).

[17] L. Zeleny, *Cereal Chem.,* **17,** 29 (1940).

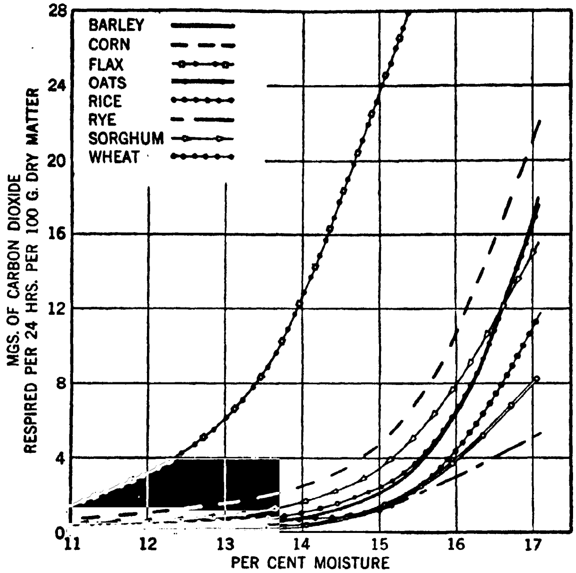


Fig. 54.—Comparative respiration of various cereal grains and flaxseed at 37.8° C. (100° F.).

After grain has matured, it goes through a sweating process; with modern methods of combine harvesting, this sweating phenomenon takes place after the grain is threshed and elevator operators regard such grain as more hazardous to store than grain at the same moisture content which has been threshed after going through the sweating process in the shock or stack.

Grain of too high a moisture content to store without serious danger of heating is normally dried at the terminal elevators by forcing heated air through thin columns or layers of the grain. A description of various types of commercial grain driers in common use has been published by the National Research Council of Canada.[18] The Council has also published an annotated bibliography of the literature dealing with the storage of grain.[19]

---

[18] National Research Council of Canada, Ottawa, Canada, *Rept.* 24 (1929).

[19] National Research Council of Canada, Ottawa, Canada, *N. R. C.* **1011** (1941).

II. CHEMICAL COMPOSITION OF THE CEREAL GRAINS

1. Average Proximate Composition of the Cereal Grains

The chemical composition of the dry matter of the different cereal grains, in common with other foods of plant origin, varies over rather wide limits, as it is influenced by genetic, soil, and climatic factors. Variations are encountered in the relative amounts of protein, lipids, carbohydrates, pigments, vitamins, and ash; the nature of the mineral elements present is also subject to wide variations.[20] However, the average proximate composition of the cereal grains given in Table 119, as compiled by Morrison[21] from a large number of individual analyses, serves as an index of their general composition. In view of the influence of climate on chemical composition, separate averages are given for barley, oats, and wheat grown in different geographical areas.

TABLE 119

AVERAGE PROXIMATE COMPOSITION OF CEREAL GRAINS[a]

	Protein, %	Fat, %	Fiber, %	N-free extract (by diff.), %	Ash, %
Barley (excluding Pacific Coast States)	11.8	2.0	5.7	68.0	2.9
Barley (Pacific Coast States)	8.7	1.9	5.7	71.0	2.6
Barley, light weight	12.3	2.3	8.5	63.7	3.0
Buckwheat	11.9	2.4	10.3	63.8	2.0
Corn, dent (well dried)	9.7	4.0	2.3	71.1	1.4
Corn, flint	9.8	4.3	1.9	71.0	1.5
Corn, pop	12.1	5.2	2.0	69.7	1.6
Corn, sweet (mature)	11.5	7.9	2.3	67.2	1.8
Oats (excluding Pacific Coast States)	12.0	4.7	10.6	60.2	3.6
Oats (Pacific Coast States)	9.0	5.4	11.0	62.1	3.7
Rice, rough[b]	8.3	1.8	8.8	64.7	5.0
Rice, brown[c]	8.9	2.0	1.0	77.2	1.1
Rye	12.3	1.7	2.3	71.7	2.0
Wheat (Great Plains area)	13.5	2.1	2.4	69.8	1.8
Wheat (Rocky Mountain States)	13.3	2.2	2.1	71.9	2.0
Wheat (Pacific Coast States)	9.9	2.0	2.7	72.6	1.9

[a] Taken by special permission of the Morrison Publishing Company, Ithaca, New York, from the extensive Appendix Tables of *Feeds and Feeding*, 20th Edition, by F. B. Morrison.

[b] Rough rice comprises the entire grain including the hulls.

[c] Brown rice consists of grain from which the hulls have been removed.

As a food group, the cereals are characterized by their relatively low protein content and their high nitrogen-free extract, which consists essentially of starch, dextrins, and sugars of which 90% or more is starch. Grains grown in the Pacific coast area with its moderate summer temperatures and humid conditions are lower in protein and higher in starch than the same grain produced in interior areas. Rice has the lowest protein content while barley, oats, wheat, and rye from the principal producing areas have somewhat more protein than dent and flint corn. The grains which have hulls, namely, barley, buckwheat, oats, and rough rice, are characterized by a relatively high crude fiber and ash content. Corn and oats contain more crude fat than the other cereals. Dent and flint corn are very similar in chemical composition while popcorn and mature sweet corn contain more protein and fat than the dent and flint types.

---

[20] K. C. Beeson, U. S. Dept. Agr., *Misc. Pub.* 369 (1941).

[21] F. B. Morrison, *Feeds and Feeding*. 20th ed., Morrison Pub. Co., Ithaca, 1940.

The various groups of components are not uniformly distributed in the different kernel structures. The hulls and bran are high in cellulose, pentosans, and ash; the germ is characterized by its high fatty oil content and is also rich in crude protein, sugars, and ash constituents. The endosperm contains the starch and is lower in protein content than the germ and also, in some cereals, than the bran; it is low in crude fat and ash constituents.

2. Factors Affecting Protein Content of Wheat and Barley

The factors influencing the protein content of wheat and barley have been very extensively studied because these cereals are grown over wide areas and their protein content is an important index of quality for the manufacture of various types of wheat and barley products. Conditions which are conducive to rapid maturation have been found to favor the production of high protein grain.[22–24] In general, regions with mild, humid climates resulting in a long growing season and slow maturation will produce starchy kernels of low protein content. Such climates are typical of insular and coastal plain areas. The climates of plateau prairies such as the Great Plains area of North America and the steppes of Russia, which are characterized by relatively hot, dry weather during the ripening of the grain, yield wheat and barley with a relatively high protein content. Within a climatic zone, seasons of high rainfall or the use of irrigation result in a general lowering of the protein level with an increase in yield. Drought conditions and the ravages of plant diseases frequently interfere with the normal filling of the kernel and affect its composition and quality. Since rainfall exerts its effect through variations in available soil moisture, it is not surprising that soils which are moisture-retentive and have a high percentage of their moisture available for the use of the plant, tend to yield grain of relatively low protein content. In fact, soil texture, which is related to moisture retention, has been found to have more influence on the chemical composition of the grain than the variations in the level of soil fertility which are normally encountered. In line with this observation, commercial fertilizers frequently markedly increase the yield of grain without greatly affecting protein content. In general, however, other factors being equal, an increase in protein content is obtained by increasing the "available nitrogen" in the soil. Thus, under similar climatic conditions, wheat grown on summer fallow or after legumes is generally of higher protein content than when grown after cereal grains or grasses; similarly the application of inorganic forms of nitrogenous fertilizers usually somewhat increases the protein content of the grain, the effect being more pronounced when the application is delayed up to the time of blossoming. Experiments reported in the literature

---

[22] C. H. Bailey, *The Chemistry of Wheat Flour.* Chemical Catalog Co., New York, 1925.

[23] *Wheat Studies, Food Research Inst.*, **10**, 229 (1934).

[24] E. G. Bayfield, Ohio Agr. Expt. Sta., *Bull.* **563** (1936).

show that phosphates applied in liberal amounts generally lower the protein content slightly while potassium-bearing fertilizers do not materially affect the composition of the grain.

As compared with the effect of environmental conditions, variety of grain is of relatively minor importance. Genetic factors are, nevertheless, involved since different varieties within a given environment produce grain of varying composition and quality. In fact, plant breeders have developed varieties which will yield grain of superior composition and quality for specific purposes; for example, wheat varieties have been created which will yield relatively high protein wheats in low-protein producing areas and corn can be raised with high or low protein or with high or low fat content.

The extent of the variations in the protein content of wheat is indicated in a survey of American hard red spring and soft red winter wheats conducted by Bailey.[25] For the crop years of 1925 to 1928, inclusive, the hard red spring wheat marketed through Minneapolis varied from somewhat less than 9 to slightly over 18% protein. Soft red winter wheats of the crop years 1932 to 1938, inclusive, varied from approximately 7 to 14% in protein content. Protein surveys of hard spring wheat and the six-row malting grades of barley produced in the Canadian prairies further exemplify the wide ranges encountered. In the 1941 crop surveys,[26] 5294 samples of wheat varied from 9.9 to 19.6% with an average of 15.1% (13.5% moisture basis) and 5601 samples of barley were found to contain from 9.3 to 15.4% protein with an average of 12.8%. Anderson and Eva[26a] have recently reviewed the literature dealing with the effect of environment factors on protein content and have summarized the data obtained in twelve annual surveys of the protein content of Western Canadian hard red spring wheat.

3. Cereal Proteins

The proteins of the cereal grains consist principally of various simple proteins; although nucleoproteins, which belong to the conjugated proteins, are found in the germ, and small amounts of proteoses, which are classified as derived proteins, are found upon fractionating the cereal proteins. The classical researches of Osborne[27, 28] have shown that the cereal grains contain water-soluble proteins (albumins), salt-soluble proteins (globulins), alcohol-soluble proteins (prolamins or gliadins), and acid- and alkali-soluble proteins (glutelins). Of these, the prolamins are characteristic of the grass family and, together with the glutelins, comprise the bulk of the proteins of the cereal grains. In maize, the prolamin called zein is the chief protein whereas oats and rice are low in prolamins, and glutelins comprise the bulk of the protein present;

---

[25] C. H. Bailey, Minn. Agr. Expt. Sta., *Tech. Bull.* **147** (1941).

[26] J. A. Anderson and W. J. Eva, *Protein Surveys of Western Canadian Wheat 1941 Crop; Protein Survey of Western Canadian Barley 1941 Crop.* Dept. of Trade and Commerce, Ottawa, Canada (1941).

[26a] J. A. Anderson and W. J. Eva, Board of Grain Commissioners for Canada, *Bull.* **4** (1943).

[27] T. B. Osborne, *The Proteins of the Wheat Kernel.* Carnegie Inst. of Washington, Washington, D. C., Pub. **84** (1907).

[28] T. B. Osborne, *The Vegetable Proteins.* Longmans, Green, New York, 1912.

TABLE 120

AMINO ACID COMPOSITION OF VARIOUS PROTEINS[a]

Source	Protein	Glycine, %	Alanine, %	Valine, %	Leucine, %	Isoleucine, %	Proline, %	Phenylalanine, %	Tyrosine, %	Serine, %	Threonine %	Methionine, %	Aspartic acid, %	Glutamic acid %	Arginine, %	Histidine, %	Lysine, %	Tryptophane, %	Cystine, %
								Plant Proteins—Cereals											
Barley	Hordein	0 0	1 4	1 4	7 0		13 7	5 5	2 4	0 1		2 2	1 3	43 2	3 2	1 3	1 0	1 0	1 5
	α Glutelin														5 6	1 1	2 9		3 1
Buckwheat	Globulin														13 0	0 6	7 9	2 7	2 5
Corn	Zein	0 0	9 8	1 9	25 0		9 0	7 6	5 9	1 0	2 2	2 3	1 8	31 3	1 8	0 8	0 0	0 2	0 8
	Glutelin	0 3			6 2		5 0	1 8	3 8				0 7	12 7	7 1	3 0	3 0		
Oats	Mixed proteins	1 0	2 5	1 8	15 0		5 4	3 2	1 5				4 0	18 4					
	Glutelin														8 3	2 3	5 0		3 0
	Gliadin											3 4			4 2	1 3	0 9	0 0	3 5
Rice	Globulin (coagulating at 74° C)								5 6						7 8	2 4	7 1	2 7	2 1
	Globulin (coagulating at 90° C)								7 5						15 2	3 0	3 6	2 3	2 9
	Glutelin		3 7		14 3		3 3	2 0	5 3				0 4	14 5	9 1	3 3	4 3	2 5	1 1
Rye	Gliadin	0 1	1 3		6 3		9 8	2 7	1 2	0 1		1 5	0 3	33 8	2 2	0 4	0 4	0 7	2 6
	Glutelin														7 1	2 7	5 4		2 6
Sorghum	Prolamin											1 8			2 2	0 6	2 6		1 1
Wheat	Gluten	0 4	0 3		4 1		4 0	1 0	1 9			2 0	0 7	24 0	4 4	1 2	2 2		
	Gliadin	0 0	2 0	3 3	6 6		13 2	2 3	3 1	0 1		2 0	0 8	43 7	2 9	1 5	0 6		1 9
	Glutenin	0 9	4 6	0 2	5 9		4 2	2 0	4 2	0 7		1 6	2 0	25 7	4 7	1 8	1 9	1 7	1 6
	Leucosin	0 9	4 4	0 2	11 3		3 2	3 8	3 3			•	3 3	6 7	5 9	2 8	2 7		
Wheat bran	Globulin								3 7			2 6			14 1	2 8	11 4	2 8	1 5
	Prolamin								3 4			1 2		•	4 4	0 8	2 4	1 4	2 3
	Albumin								4 2		•				10 0	2 6	4 5	4 8	3 3

Plant Proteins—Other than Cereals

Adsuki bean	α-Globulin	..	...	...	..	...	..	...	2.1	...	...	...	...	..	5.4	2.2	8.3	1.7	1.8
	β-Globulin	..	...	...	..	...	..	...	...	...	...	...	...	..	7.0	2.5	8.4	1.0	0.01
Cottonseed	Total globulin	1.2	4.5	Present	15.5	...	2.3	3.9	2.3	0.4	...	...	2.9	17.6	13.5	3.5	4.3	2.6	1.1
	α-Globulin	..	...	...	..	...	..	...	...	...	...	...	...	..	13.0	3.5	3.9	...	0.8
	β-Globulin	..	...	...	..	...	..	...	...	...	...	...	...	..	13.2	4.0	4.0	...	0.8
Cow pea	Globulin	0.0	1.0	0.3	7.8	...	5.2	5.3	2.3	...	...	...	4.0	16.9	7.2	3.1	4.3	1.6	0.5
Lima beans	α-Globulin	..	...	...	..	...	..	...	...	...	...	...	...	..	5.7	3.7	7.8	1.9	1.6
	β-Globulin	..	...	...	..	...	..	...	...	...	...	...	...	..	5.1	2.6	8.5	2.2	0.1
	Albumin	..	...	...	..	...	..	...	...	...	...	...	...	..	5.7	5.5	6.0	1.4	1.1
Mung bean	α-Globulin	..	...	...	..	...	..	...	...	...	...	...	...	..	5.1	3.3	6.1	2.0	1.7
	β-Globulin	..	...	...	..	...	..	...	...	...	...	...	...	..	7.5	2.0	9.1	1.2	0.04
Navy bean	Phaseolin	0.6	1.8	1.0	9.7	...	2.8	3.3	2.2	0.4	...	...	5.3	14.6	6.1	2.6	4.6	0.9	0.6
	Comphaseolin	..	...	...	..	...	..	...	...	...	...	...	...	..	6.9	0.8	10.7	2.8	1.5
Pea	Legumin	0.4	2.1	?	8.0	...	3.2	3.7	1.5	0.5	...	...	5.3	17.0	11.7	1.7	5.0	1.8	0.8
	Legumelin	0.5	0.9	0.7	9.6	...	4.0	4.8	1.6	...	...	...	4.1	13.0	5.4	2.3	3.0	...	...
	Vicilin	0.0	0.5	0.1	9.4	...	4.1	3.8	2.4	?	...	...	5.3	21.3	8.9	2.2	5.4	0.1	0.6
Peanut	Arachin	0.0	4.1	1.1	3.9	...	1.4	2.6	5.5	5.2	2.6	0.5	5.6	19.5	13.5	1.9	5.0	0.9	1.5
	Conarachin	..	...	...	..	...	..	...	...	...	2.0	3.0	...	..	14.6	1.8	6.0	2.1	4.4
Potato	Tuberin	..	4.9	1.1	12.2	...	3.0	3.9	4.3	...	...	...	...	4.6	4.2	2.3	3.3	...	4.4
Soybean	Glycinin	1.0	...	0.7	8.5	...	3.8	3.9	1.9	...	...	1.8	9.4	19.5	8.1	1.4	9.1	1.7	1.1
Velvet bean	Stizolobin	1.7	2.4	2.9	9.0	...	4.0	3.1	6.2	0.7	...	1.9	9.2	14.6	7.1	8.3	8.5	1.4	1.5

Animal Proteins

Chicken	Muscle	0.7	2.3	?	11.2	...	4.7	3.5	2.2	?	...	...	3.2	16.5	6.5	2.5	7.2	...	...
Eggs	Ovalbumin	0.0	2.2	2.5	10.7	...	3.6	5.1	4.0	?	...	...	6.2	13.3	4.9	1.7	3.8	2.2	0.9
	Conalbumin	..	...	...	..	...	..	...	...	...	...	...	...	..	..	...	..	5.0	3.4
	Vitellin	1.1	0.7	1.9	9.9	...	4.2	2.5	3.4	?	...	...	2.1	12.9	7.5	1.9	4.8	8.4	0.8
Fish (halibut)	Muscle	0.0	...	0.8	10.3	...	3.2	3.0	2.4	...	...	...	8.0	13.7	6.3	2.5	7.5	1.2	1.3
Gelatin	Gelatin	25.5	8.7	0.0	7.1	0.0	9.5	1.4	Trace	0.4	...	...	3.4	5.8	8.2	0.9	5.9	0.0	Trace
Milk	Casein	0.4	1.8	7.9	9.7	...	7.6	3.9	6.5	0.5	...	...	4.1	21.8	3.8	2.5	7.6	2.2	0.2
	Lactalbumin	0.4	2.4	3.3	14.0	...	3.8	1.2	1.9	1.8	...	...	9.3	12.9	3.5	2.6	9.9	2.7	2.8
Ox	Muscle	2.1	3.7	0.8	11.6	...	5.8	3.1	2.2	...	...	...	5.9	15.5	7.5	1.8	7.6	1.2	1.5
Salmon	Salmine	..	+	4.3	+	...	11.0	...	...	...	...	...	...	..	87.4	...	..	...	...
Scallop	Muscle	0.0	...	...	8.8	...	2.3	4.9	1.9	...	...	...	3.5	14.9	7.4	2.0	5.8	...	...
Shrimp	Muscle	..	...	...	..	...	..	...	4.9	...	...	...	7.0	15.0	10.2	3.8	7.6	1.2	1.7

[a] Prepared by D. Breese Jones, Protein and Nutrition Research Division, Bureau of Agricultural Chemistry and Engineering (private communication, 1942).

the glutelin of oats is called avenin and the glutelin of rice, oryzenin. Wheat, rye, and barley are intermediate between corn, on the one hand, and oats and rice, on the other, in regard to the relative proportions of prolamins and glutelins. The prolamins of wheat and rye are called gliadin while the prolamin of barley is called hordein. The glutelin of wheat is called glutenin. See page 111.

The various proteins are not distributed uniformly in the kernel. Thus, the proteins fractionated from the inner endosperm of wheat consist chiefly of a prolamin, gliadin, and a glutelin, glutenin, in apparently approximately equal amounts; the embryo proteins consist of nucleoproteins, an albumin (leucosin), a globulin, and proteoses while in wheat bran, a prolamin predominates with lesser quantities of albumins and globulins.[29] The wheat endosperm proteins, gliadin and glutenin, form a tenacious colloidal complex, known as gluten, when water is added; this unique property is responsible for the superiority of wheat over the other cereals for the manufacture of leavened products as it makes possible the formation of a dough which will retain the carbon dioxide produced by yeast or chemical leavening agents. The gluten proteins collectively contain about 17.55% nitrogen; hence, in estimating the crude protein content of wheat and wheat products from determinations of total nitrogen, the factor 5.7 is normally employed rather than the customary value of 6.25 which is based on the assumption that, on the average, proteins contain 16% nitrogen.

In recent years there has been much discussion of the adequacy of solubility and precipitation methods as bases for protein classification and serious doubts have been expressed as to whether such methods of separation result in the isolation of individual proteins of definite chemical composition.[30, 31] Many investigations have been conducted in an effort to relate the breadmaking quality of wheat flours with the relative proportions of gliadin and glutenin and with variations in their chemical composition.[32] Much conflicting evidence has been published but it is now generally recognized that the breadmaking "quality" of the wheat flour proteins is more closely related to their colloidal properties than with such variations in chemical composition as can be determined by present methods.

From the nutritive standpoint, however, the chemical composition of the proteins is of great importance. Animal feeding experiments with individual proteins or the proteins from a single source have revealed important differences in the nutritive values of various food proteins. As a class, the cereal proteins are not so high in biological value as those of certain of the legumes, of nuts, or of animal products. That these differences in biological value are related to amino acid composition has been shown by the results of feeding experiments in which the individual proteins have been supplemented with certain amino acids. Thus, zein (the prolamin of corn) which is lacking in lysine and low in tryptophane, will not even maintain life in the rat; when supplemented with these amino acids and fed at a sufficiently high level, it will maintain the nitrogen equilibrium of the rat and serve as the sole source of protein in the diet. Similarly, the wheat endosperm proteins are poor in lysine and tryptophane and the addition of these amino acids greatly improves their nutritional value. The bran proteins contain larger percentages of lysine and tryptophane, and nutritional experiments show that the whole-wheat proteins are superior to the endosperm proteins (or to white flour).[33]

---

[29] D. B. Jones, *Cereal Chem.*, **14**, 771 (1937).

[30] R. A. Gortner, *Outlines of Biochemistry.* 2nd ed., Wiley, New York, 1938, p. 417.

[31] A. G. McCalla, and R. C. Rose, *Can. J. Research*, **12**, 346 (1935).

[32] P. F. Sharp, and R. A. Gortner, Minn. Agr. Expt. Sta., *Tech. Bull.* **19** (1923).

It must be emphasized that several of the amino acids present in animal proteins can be formed in the animal body: other amino acids cannot be synthesized by the animal and hence are essential or indispensable components of the foods. In the instance of the rat and dog, ten of the amino acids are "essential," namely, arginine (essential for optimal growth but not for maintenance), histidine, isoleucine, leucine, lysine, methionine (cystine may supplement a limited supply), phenylalanine, threonine, tryptophane, and valine. The specific amino acids which must be supplied in the food vary somewhat for different animal species; research is now in progress to ascertain the precise amino acid requirements of humans.

In view of the importance of the chemical composition of proteins in relation to their biological value, much effort has been expended in the extremely difficult task of determining the kind and quantities of the various amino acids present in different proteins. Although the data are fragmentary, a compilation of the amino acid determinations which have been made on several proteins is given in Table 120, see pp. 618–619. The results of experimental feeding trials with individual cereal proteins have frequently been interpreted as proof of their low nutritional value; but the data recorded in Table 120 illustrate that one protein may be supplemented by another in the same grain. While it is true that the biological values of the proteins of entire cereal grains is greater than those of the refined mill products which consist chiefly of the endosperm, it must be emphasized that the American diet normally comprises various cereals as well as animal products. Under these conditions, the different proteins tend to supplement each other, and the cereals are important and valuable sources of amino acids for the synthesis of the body proteins.

4. Cereal Carbohydrates

While starch is the principal polysaccharide present in the cereals, small quantities of dextrins are usually found in fractionation studies; these may result, in considerable measure, from the action of amylases on starch which has been mechanically injured during the grinding of the cereal. Pentosans are found principally in the bran tissues, associated with cellulose and lignin. Sucrose is the principal sugar present and is found in the greatest amounts in the germ tissues; small amounts of a trisaccharide sugar (raffinose) have been reported as present in several of the cereals.

5. Cereal Lipids

The cereal lipids are located largely in the embryo and consist of fatty oils, phosphatides (such as lecithin), and unsaponifiable material, such as the sterol isomers known as sitosterol. See page 100. The unsaponifiable fraction of wheat germ oil (and, to a much lesser extent, rice germ oil) is particularly high in the tocopherols which possess vitamin E activity. The fatty oils of cereals consist largely of glycerides of oleic and linoleic acids as the commonly found components. The iodine value of the cereal oils ranges from about 100 to 125 so that they are all semidrying oils.

---

[33] A. M. Copping, *Nutrition Abstracts & Revs.*, 8, 555 (1939).

6. Enzymes

The cereal grains contain many enzymes which catalyze chemical changes that occur during growth, ripening, processing, and storage. Of these, only the enzymes involved in the hydrolytic cleavage of starch and proteins will be considered here. It is now recognized that at least two enzymes, α-amylase and β-amylase, are involved in starch degradation. α-Amylase will act on both raw and gelatinized starch; starch pastes subjected to the action of α-amylase preparations are rapidly liquefied and converted to short-chain dextrins which do not give a color with iodine; maltose is produced slowly and the reaction can be pictured as involving a series of "center cleavages." β-Amylase, on the other hand, is predominantly responsible for maltose formation from gelatinized starch and dextrins; the ability to form colored complexes with iodine is lost slowly and hence it appears that this enzyme splits off maltose from the ends of the chains.[34, 35] The combined action of α- and β-amylase results in very rapid saccharification; this is explained as being due to a great increase in the number of points of attack for β-amylase as a result of the action of α-amylase. Ungerminated wheat, barley, and rye have considerable β-amylase activity but exhibit little or no α-amylase activity, upon germination, β-amylase activity increases to a maximum in a few days, whereas α-amylase activity develops more slowly and increases throughout the germination period.[36, 37]

At the beginning of germination, marked cytological changes occur in the epithelial cells of the scutellum; these cells greatly enlarge and the protoplasm assumes a granular nature. These changes are soon followed by "solution" of the cell walls of the endosperm and hydrolysis of the endosperm reserves adjacent to the scutellar epithelium begins; the scutellar epithelium thus seems to synthesize enzymes, such as cytase, proteolytic enzymes, and amylases. It has been found, however, that, when ungerminated grain is digested with water containing the proteolytic enzyme, papain, there is an increase in β-amylase activity comparable to that which takes place upon germination. Accordingly, today, the view is held that germination brings about a liberation of "bound" β-amylase, which is present in the grain, by the action of the proteolytic enzymes which are simultaneously developed during germination. The saccharogenic activity exhibited by germinated grain depends upon the combined action of the β-amylase (that originally present in the free state plus that liberated through proteolytic action during germination) and the α-amylase formed or activated

---

[34] C. S. Hanes, *New Phytologist*, **36**, 7 (1937).

[35] C. M. Hollenbeck and M. J. Blish, *Cereal Chem.*, **18**, 754 (1941).

[36] E. Kneen, B. S. Miller, and R. M. Sandstedt, *Cereal Chem.*, **19**, 11 (1942)

[37] E. Kneen, O. C. Beckord, and R. M. Sandstedt, *Cereal Chem.*, **18**, 741 (1941).

upon germination. It has been found that the amylase activity of malt is highly positively correlated with the nitrogen content of the barley from which it is made.[38–40]

The proteolytic enzymes of the cereal grains, especially wheat, barley, and rye, have been extensively studied. In the germination of barley and other grains, there is a close parallelism between the intensity of respiration and the increase in the rate of starch and protein degradation; a considerable proportion of the protein is converted into soluble derived proteins during the brewing process. Variety and environmental factors influence the extent of the modification which occurs under fixed conditions of growth.[41, 42] Jorgensen[43] and Balls and Hale[44] have shown that the proteinases of wheat and wheat flour are of the papain type, as indicated by the inhibitory action of oxidizing agents, such as bromates and persulfates, and the powerful activating effect of sulfhydryl compounds. The germ is relatively rich in proteinases, as well as glutathione. Some workers have attributed the harmful effect of wheat germ upon the baking quality of flour to increased proteolytic activity, others do not believe that proteolytic activity plays a very important role in the baking quality of flours milled from sound wheat and are inclined to the view that the deleterious effects of wheat germ and the influence of oxidizing and reducing agents on flour-baking properties are associated with oxidation-reduction phenomena in which sulfhydryl compounds play a significant part.[45, 46]

7. Vitamins of the Cereal Grains

All the cereal grains contain vitamins of the B group but they are completely lacking in vitamin C (unless the grain is sprouted) and vitamin D. Yellow corn differs from white corn and the other cereal grains in containing carotenoid pigments (principally cryptoxanthin, with smaller quantities of carotenes) which are convertible in the body to vitamin A. A compilation of numerous assays shows the vitamin A activity of yellow corn to vary between 200 and 800 I. U. per 100 g. Wheat also contains carotenoids but these consist practically entirely of xanthophyll which is not a precursor of vitamin A.[47] The oils of the embryos of cereal grains, particularly wheat, are rich sources of vitamin E.[48] As the vitamin E requirements of man and the larger animals have not been well established, interest in the cereals as sources of vitamins centers around the various members of the B complex.

The recognition of the importance of the B vitamins in nutrition has created wide-

---

[38] L. R. Bishop, *J. Inst. Brewing*, **36**, 421 (1930); **40**, 75 (1934); **42**, 103 (1936).

[39] J. A. Anderson, C. A. Ayre, and W. O. S. Meredith, *Can. J. Research*, **17**, 25 (1939).

[40] J. A. Anderson, H. R. Sallans, and W. O. S. Meredith, *Can. J. Research*, **C19**, 278 (1941).

[41] R. H. Hopkins, and C. B. Krause, *Biochemistry Applied to Malting and Brewing*. Van Nostrand, New York, 1937.

[42] J. G. Dickson, A. D. Dickson, H. L. Shands, and B. A. Burkhart, *Cereal Chem.*, **15**, 133 (1938).

[43] H. Jorgensen, *Cereal Chem.*, **13**, 346 (1936).

[44] A. K. Balls, and W. S. Hale, *Cereal Chem.*, **15**, 622 (1938).

[45] B. Sullivan, M. Howe, F. D. Schmalz, and G. R. Astleford, *Cereal Chem.*, **17**, 507 (1940).

[46] T. Shen, and W. F. Geddes, *Cereal Chem.*, **19**, 609 (1942).

[47] L. Zechmeister, and R. B. Escue, *Proc. Natl. Acad. Sci. U. S.*, **27**, 528 (1941).

[48] D. S. Binnington, and J. S. Andrews, *Cereal Chem.*, **18**, 678 (1941).

spread interest in their distribution in the cereal grains and in the influence of milling processes on the vitamin levels of refined cereal products manufactured for human consumption. Animal assay methods did not lend themselves to the accurate determination of relatively low vitamin levels but the recent development of reliable chemical and microbiological assay methods has made it possible to carry out extensive vitamin surveys of the cereal grains and their products.

The results of two surveys of the thiamine content of cereals (entire grain), summarized in Table 121, show that barley, corn, rye, wheat, and the grain sorghums have rather similar average thiamine values, whereas oats and buckwheat contain somewhat more, and rice somewhat less, of this vitamin. The thiamine content of each cereal varies rather widely and is influenced by the environmental conditions under which the grain is grown and to a less extent by variety. Hard red spring wheats showed the highest average thiamine content followed by hard red winter, West Coast white wheats and Ohio soft wheats in the order mentioned. Durum wheats apparently are similar in thiamine content to hard red spring wheats grown in the same location. Similar relations between the relative thiamine contents of the different classes of American wheat have been obtained by Downs and Cathcart.[49] In a survey of Western Canadian hard red spring wheats, the thiamine values ranged from 2.2 to 8.0, with an average of 3.93 γ per g.[50]

TABLE 121

THIAMINE CONTENT OF CEREAL GRAINS

Cereal	Data of Nordgren and Andrews[a] Thiamine in γ per g.				Data of Schultz, Atkin, and Frey[b] Thiamine in γ per g.				Data of Kik[c] Thiamine in γ per g.			
	No. of samples	Mean	Range		No. of samples	Mean	Range		No. of samples	Mean	Range	
			Min.	Max.			Min.	Max.			Min.	Max.
Barley	6	6.50	5.69	7.34	37	6.2	3.8	9.2	..	..	..	..
Buckwheat	..	..	..	..	5	7.2	4.2	8.5	..	..	..	..
Corn, yellow	6	5.38	4.83	6.22	..	...	...	..	..	..	..	..
Corn, white	6	6.09	4.94	6.70	..	...	...	..	..	..	..	..
Corn, all analyses	12	5.74	4.83	6.70	23	5.3	4.1	8.0	..	..	..	..
Oats	6	9.26	8.11	10.80	21	7.2	4.8	10.3	..	..	..	..
Rice, rough	..	..	..	..	..	...	...	..	7	2.93	2.60	3.07
Rice, brown, whole	..	..	..	..	..	...	...	..	7	3.02	2.58	3.51
Rye	6	4.65	4.15	5.03	10	4.8	4.0	5.7	..	..	..	..
Sorghums, grain	7	5.91	4.26	8.75	..	...	...	..	..	..	..	..
Wheat, hard red spring:												
Minnesota	24	6.06	4.43	7.70	..	...	...	..	..	..	..	..
Canada	13	5.09	3.20	6.20	..	...	...	..	..	..	..	..
Wheat, hard red winter	35	4.78	3.64	5.98	..	...	...	..	..	..	..	..
Wheat, West Coast	12	4.61	3.88	5.38	..	...	...	..	..	..	..	..
Wheat, soft (Ohio)	15	4.48	3.95	5.25	..	...	...	..	..	..	..	..
Wheats, all	99	5.06	3.20	7.70	33	5.6	4.2	7.3	..	..	..	..

[a] R. Nordgren and J. S. Andrews, *Cereal Chem.*, **18**, 802 (1942).
[b] A. S. Schultz, L. Atkin, and C. N. Frey, *Cereal Chem.*, **18**, 106 (1941).
[c] M. C. Kik, *Cereal Chem.*, **20**, 103 (1943). Results are expressed on a dry matter basis.

The vitamin B_2 or riboflavin contents of barley, corn, oats, and three classes of common wheat, as determined by Andrews, Boyd, and Terry,[51] are recorded in Table 122.

---

[49] D. E. Downs and W. H. Cathcart, *Cereal Chem.*, **18**, 796 (1941).

[50] H. Johannson and C. E. Rich, *Cereal Chem.*, **19**, 308 (1942).

[51] J. S. Andrews, H. M. Boyd, and D. E. Terry, *Cereal Chem.*, **19**, 55 (1942).

In a study of 18 varieties of rice grown at various localities in Arkansas, California, Louisiana, and Texas, Kik and Van Landingham[51a] found that the riboflavin content of the rough rice ranged between 0.43 and 0.83 γ per g. (dry basis). These data show that the cereals are much poorer sources of riboflavin than they are of thiamine. The variation between and within cereals is much more limited and, in contrast with thiamine, the data at present available indicate that the riboflavin levels of wheat are not appreciably influenced by environment, but significant differences are found between varieties.

Information on the nicotinic acid (niacin) content of cereals is less extensive than for thiamine and riboflavin and at present there is some uncertainty as to the suitability of the available analytical methods for the nicotinic acid assay of cereals.[52-54] Chromogenic substances interfere with the application of colorimetric procedures and most workers in this field employ the microbiological method originally described by Snell and Wright.[55] With cereals, however, the nicotinic acid values obtained depend upon the procedure employed in extracting the sample; alkaline extraction gives values varying up to about twice those obtained with water alone. Employing alkaline hydrolysis, Tepley, Strong, and Elvehjem[53] found the following nicotinic acid values for cereal grains: barley 47, buckwheat 44, corn 16 to 26, oats 11 to 16, rice (brown) 69, rye 9 to 13, and wheat 50 to 70 γ per g. While based on only a few samples, these results indicate that the cereals vary considerably in their nicotinic acid content. Wheat, buckwheat, barley, and rice contain much higher levels than corn, oats, and rye. Kik and Van Landingham[54a] obtained niacin values of 39.3 to 59.0 γ per g. (mean 46.5 γ per g.) dry basis, for 23 varieties of rough rice grown in four States. Tepley, Strong, and Elvehjem[54] have conducted a survey of the nicotinic acid, pantothenic and pyridox-

TABLE 122

RIBOFLAVIN CONTENT OF CEREAL GRAINS[a]

Cereal	Riboflavin in per g.			
	No of varieties	Mean	Range	
			Min.	Max.
Barley	6	1.21	1.05	1.50
Corn, yellow	13	1.40	1.30	1.50
Corn, white	5	1.38	1.30	1.50
Oats	5	1.30	1.10	1.45
Rye	6	1.43	1.30	1.65
Wheat, spring	6	1.20	1.06	1.32
Wheat, winter	4	1.18	1.00	1.30
Wheat, soft	15	1.17	1.00	1.30

[a] J. S. Andrews, H. M. Boyd, and D. E Terry, *Cereal Chem.*, **19,** 55 (1942).

[51a] M. C. Kik and F. B. Van Landingham, *Cereal Chem.*, **20,** 560 (1943).

[52] J. S. Andrews, H. M. Boyd, and D. E. Terry, *Ind. Eng. Chem., Anal. Ed.*, **14,** 663 (1942).

[53] L. J. Tepley, F. M. Strong, and C. A. Elvehjem, *J. Nutrition*, **23,** 417 (1942).

[54] L. J. Tepley, F. M. Strong, and C. A. Elvehjem, *J. Nutrition*, **24,** 167 (1942)

[54a] M. C. Kik and F. B. Van Landingham, *Cereal Chem.*, **21,** in press.

[55] E. E. Snell and L. D. Wright, *J. Biol. Chem.*, **139,** 675 (1941).

ine content of 59 wheat samples of varying types and origins. These results, summarized in Table 123, show that the nicotinic acid and pyridoxine levels of the various classes of wheat fall in the same general range but that the soft red winter wheats from Ohio averaged lower in pantothenic acid content than the others.

The distribution of the vitamins of the B complex in the various kernel structures is not uniform, although the endosperm invariably contains the lowest levels. It has been estimated that, of the total thiamine in the wheat kernel, the endosperm contains 24%, the embryo 15%, and the outer layers about 61%.[56] Riboflavin is similarly distributed but a somewhat higher proportion of the total is found in the endosperm.[52] In the instance of riboflavin, the germ has the richest concentrations, whereas it is relatively low in nicotinic acid; the bran is the richest kernel structure in nicotinic acid.

TABLE 123

NICOTINIC ACID (NIACIN), PANTOTHENIC ACID, AND PYRIDOXINE CONTENTS OF WHEAT

Type	Vitamin values in γ per g.									
	No. of samples	Niacin			Pantothenic acid			Pyridoxine		
		Mean	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.
Hard red spring	11	66	48	106	13.7	9.8	17.0	4.5	3.6	5.6
Hard red winter	30	57	47	66	14.3	9.1	17.5	4.5	3.2	6.1
Hard white spring	4	55	53	57	13.2	11.3	15.2	4.8	4.4	5.5
Soft red winter (Kawvale variety)	3	55	52	59	15.1	14.4	15.7	4.9	3.9	6.3
Soft red winter (from Wooster)	6	60	52	67	9.8	9.3	10.6	4.6	4.2	4.8
Soft white spring	1	56	..	...	11.9	..	..	4.9	...	...
Soft white winter	3	62	59	65	11.1	10.9	11.4	4.7	4.3	5.0
White club	1	56	..	...	11.4	..	..	4.5	...	...
All wheats	59	59	47	106	13.4	9.3	17.5	4.6	3.2	6.3

8. Mineral Constituents of Cereal Grains

In ashing biological materials, the mineral elements present in both organic and inorganic combination are converted into the inorganic compounds which comprise the ash; during ignition some of the mineral elements, such as sulfur, chlorine, and certain of the minor elements, may partly escape as volatile compounds. In the ash of naked cereals, potassium, phosphorus, and magnesium predominate, followed by sulfur, chlorine, calcium, and sodium. The ash of covered cereals such as barley and oats and rice is relatively high in silicon because of the high silica content of the hulls. In addition to these major elementary constituents, several minor, or trace elements, are present in much smaller but more widely varying amounts; these include aluminum, arsenic, boron, bromine, cobalt, copper, iodine, iron, lithium, nickel, selenium, tin, and titanium. Beeson[57] has recently compiled the available mineral analyses of plant products and discussed the factors influencing the wide variations which have been found, particularly in the concentrations of the trace elements.

---

[56] R. C. Sherwood, R. Nordgren, and J. S. Andrews, *Cereal Chem.*, **18**, 811 (1941).

[57] K. C. Beeson, U. S. Dept. Agr., *Misc. Pub.* **369** (1941).

The mean elementary composition of entire cereal grains in respect to certain selected mineral elements, as compiled by Beeson,[57] is given in Table 124. As a group, the cereals are low in the nutritionally important element, calcium, and, as will be shown later, its concentration and that of other ash constituents is greatly reduced by the milling processes to which the cereals are subjected in the preparation of refined foods. In these processes, the hulls, germ, and bran, which are the structures rich in minerals and vitamins, are more or less completely removed; oats constitute a partial exception since essentially only the hull is removed.

TABLE 124

MEAN PERCENTAGES OF SELECTED MINERAL ELEMENTS IN CEREAL GRAINS[a]

	Ca, %	Mg, %	K, %	Na, %	P, %	Cl, %	S, %	Fe, %
Barley	0.064	0.14	0.55	0.07	0.41	0.17	0.17	0.0099
Buckwheat	...	..	..	..	0.33	...	..	0.0034
Corn	0.015	0.16	0.40	0.05	0.43	0.024	0.14	0.0037
Oats	0.100	0.16	0.48	0.09	0.34	1.38	0.19	0.0203
Rice, rough	0.09	0.16	0.38	0.09	0.36	...	..	0.0076
Rye	0.115	0.14	0.53	0.04	0.37	0.02	0.18	0.0057
Wheat	0.050	0.17	0.48	0.10	0.40	0.09	0.18	0.0065

[a] Abstracted from data compiled by K. C. Beeson, U. S. Dept. Agr., *Misc. Circ.* **369** (1941).

The phosphorus content of the different cereals is very similar and is several times higher than that of calcium. A considerable part of the phosphorus is in the form of phytin, the calcium or magnesium salt of phytic acid (inositol hexaphosphoric acid), and there is some question as to the availability of phosphorus in this form; moreover, the availability of cereal calcium is stated by some workers to depend upon the amount of phytic acid present. Nutritional authorities generally recommend a calcium-phosphorus ratio of 1 or 2:1 so that the ratios existing in the cereal grains are undesirably low. Rye and oats are the highest in calcium of the various cereal grains while oats are particularly high in iron.

III. BARLEY

1. Utilization[58]

In the United States, barley is predominantly a feed grain, but a considerable percentage of the crop is used for the production of malt. Relatively smaller quantities are employed in the manufacture of pot and pearl barley, barley grits, and barley flour. During the period 1934 to 1939, inclusive, an average of 64,791,500 bu., or 22% of the average annual barley production, was used for malting; in recent years the production of malting barley and malt has somewhat increased. The major uses of barley are shown in Table 125.

[58] Contributed by J. G. Dickson, Professor of Plant Pathology, University of Wisconsin.

TABLE 125

MAJOR USES OF BARLEY

- *Pearling*
  - Soups
  - Sage dressings
- *Feed*
  - Poultry
  - Hogs
  - Cattle
- *Export*
  - Feed
  - Malt
- *Malting*
  - Brewing
    - Beverages
    - Feeds
      - Dried grains
      - Wet grains
  - Export
  - Coffee substitutes
  - Malt flour
  - Yeast
    - Home
    - Bakeries
    - Medicinal
  - Malted milk concentrates
    - Malted milks
    - Infant foods
  - Malt sirups
    - Candies
    - Household
      - Baking
      - Candies
      - Beverages
    - Bakeries
      - Bread
      - Cookies
      - Cakes
    - Textile industry
    - Medical
      - Laxative
      - Fevers
      - Inflammations
    - Breakfast cereals
  - Distilleries
    - Whiskies, Gins
    - Alcohol
    - Medicinal Spirits
    - Vinegar
  - Caramel and black malts
    - Breakfast cereals
    - Sugar colorings
  - Malt sprouts
    - Yeast
    - Vinegar
    - Dairy feeds

2. Chemical Composition

The average proximate composition, vitamin and mineral content of pearled barley, and barley flour are given in Tables 126 and 127. Proximate analyses of barley feeds are shown in Table 129.

IV. BUCKWHEAT

1. Utilization

Approximately two-thirds of the average annual buckwheat crop of 7,315,000 bu. is used on the farms where it is produced. In 1939, 783,575 bu. were ground for livestock feeding or milled into groats, grits, or flour. The major portion of the buckwheat which is processed for human food is milled into flour and in 1939, 22,639,600 lbs. were produced. It is not as finely ground or as highly refined as wheat or rye flour and is used mainly as a pancake flour. Ready-prepared mixtures are also made con-

TABLE 126

PROXIMATE COMPOSITION AND FUEL VALUE OF CEREAL PRODUCTS, AS PREPARED FOR HUMAN CONSUMPTION[a]

	Moisture, %	Protein, %	Fat, %	Fiber, %	Total carbo-hydrates, %	Ash, %	Fuel value per 100 g., cal.
Barley, flour	10.0	10.2	1.7	0.7	76.9	1.2	364
Barley, pearled	10.8	8.7	1.0	0.8	78.3	1.2	357
Buckwheat, flour, light	12.0	6.3	1.1	0.4	79.7	0.9	354
Buckwheat, flour, dark	12.0	12.4	2.4	1.0	71.6	1.6	358
Corn meal, whole, white or yellow	12.0	9.1	3.7	2.0	73.9	1.3	365
Corn meal, degerminated, white	12.0	7.5	1.1	0.8	78.8	0.6	355
Corn meal, degerminated, yellow	12.0	8.3	1.2	0.7	78.0	0.5	356
Corn flakes	9.3	7.9	0.7	0.5	80.3	1.8	359
Corn flour	12.1	7.9	2.2	0.7	77.0	0.8	359
Corn hominy	11.4	8.5	0.8	0.4	78.9	0.4	357
Oat meal or rolled oats	8.3	14.2	7.4	1.2	68.2	1.9	396
Macaroni products:							
Macaroni	11.0	13.0	1 4	0.4	73.9	0.7	360
Noodles, egg	9.1	14.3	5.0	0.3	70.6	1.0	385
Rice, brown	12 0	7.5	1.7	0.6	77.7	1.1	356
Rice, white	12.3	7.6	0.3	0.2	79.4	0.4	351
Rice, puffed	9.3	6.7	0.3	0.3	83.3	0.4	363
Rice, flakes	8.3	7.7	0.5	0.7	82.0	1.5	363
Rice, flour	12.1	7 4	0.5	0.4	79.5	0.5	352
Rice, wild	8 6	13.8	0 8	1.4	75.5	1.3	364
Rye flour, light	11.0	8.9	0.9	1.1	78.5	0.7	358
Rye flour, medium	11.0	11.0	1.2	1.5	75.8	1.0	358
Rye flour, dark	11.0	14.9	2.1	2.1	70.0	2.0	358
Wheat flour, graham:							
Hard red wheat	11.0	13 9	2.1	2.3	71.4	1.6	360
Soft red wheat	11.0	11.4	2.0	2.3	73.9	1.7	359
White wheat	11.0	10.7	2.0	1.9	74.6	1.7	359
Wheat flour, white:							
Straight grade:							
Hard red	12.0	12 0	1.2	0.4	74.3	0.5	356
Soft red	12.0	10.8	1.0	0.4	75.7	0.5	355
White	12.0	9.3	1.0	0.4	77.2	0.5	355
Patent:							
All-purpose	12.0	10.8	0.9	0.3	75.9	0.4	355
Bread	12.0	11.8	1.1	0 3	74.6	0.5	356
Cake or pastry	12.0	8.3	0.8	0.2	78.5	0.4	354
Self-rising (salt and leavening added):							
Patent, soft	12.0	7.9	0 8	0.2	75.4	3.9	340
Straight	12.0	10.2	0.9	0.4	72.9	4.0	340
Clear	12.0	10.9	1.4	0.4	71.6	4.1	343
Prepared, shortening added	9.5	8.3	11.9	0.3	66.3	4.0	406
Pancake, from wheat and mixed cereals	11.2	10.3	1.5	0.6	72.3	4.7	344
Wheat breakfast foods:							
Bran flakes, bran with other parts of grain	6.2	13.0	1.9	3.1	74.9	4.0	369
Farina	11.0	11.5	1.0	0.3	76.1	0.4	359
Puffed wheat	7.8	13.4	1.7	1.8	75.6	1.5	371
Shredded wheat	7.7	10.4	1.4	2.1	78.7	1.8	369
Wheat flakes	4.7	10.4	1.3	1.6	79.9	3.7	373
Wheat meals, ground, cut or cracked grain	8.7	11.7	2.0	1.8	75.8	1.8	368
Wheat bran, crude	10.1	16.6	3.7	10.3	63.5	6.1	354
Wheat bran, packaged almost wholly bran	7.4	15.9	4.2	8.4	66.2	6.3	366
Wheat germ, commercially milled, contains some bran and flour	11.0	25.2	10.0	2.5	49.5	4.3	389

[a] Abstracted from tables compiled by C. Chatfield and G. Adams, U. S. Dept. Agr., *Circ.* 549 (1940).

Table 127

Selected Mineral and B Complex Vitamin Content of Cereal Foods[a]

Cereal	Mineral elements, %				Vitamins in γ per g.		
	Ca	P	Fe	Cu	Thiamine	Riboflavin	Niacin
Barley, pearled	0.020	0.181	0.0020	0.00040	0.50	0.10	30
Buckwheat flour	0.010	0.176	0.0012	0.00070	...	..	...
Corn meal, yellow, whole	0.018	0.190	0.0009	0.00020	2.30	0.80	10
Corn, hominy, white	0.011	0.144	0.0009	.....	1.80	..	...
Oat meal	0.069	0.392	0.0038	0.00050	8.10	1.50	10
Rice, brown	0.084	0.290	0.0020	0.00036	3.00	1.50	70
Rice, white (polished)	0.009	0.096	0.0009	0.00019	0.20	0.80	10
Rye flour	0.018	0.289	0.0013	0.00042	1.80	0.60	Data insuff.
Wheat, entire	0.045	0.423	0.0043	0.0006	5.20	1.20	50
Wheat flour, white	0.020	0.092	....	0.00017	0.87	0.40	10
Wheat flour enriched, min.[b]	0.110	...	0.0013	.....	3.70	2.20	10
Wheat flour enriched, max.[b]	0.441	...	0.0053	.....	5.55	3.30	20
Wheat bran	0.120	1.215	0.0126	0.00117	5.20	3.50	320
Wheat germ	0.070	1.050	0.0086	0.00127	35.00	7.00	50
Macaroni	0.022	0.144	0.0012	0.00007	1.3	..	20

[a] Abstracted from *Nutritional Charts.* 11th ed., H. J. Heinz Co., Pittsburgh, Pa., 1942.

[b] These data are for enriched wheat flour prepared according to the enrichment standards which were in effect from January 1, 1942, to September 30, 1943. (The riboflavin requirement of these standards was not made effective.) For the calcium, iron, and B vitamin contents of enriched flour made according to the current standards effective October 1, 1943, see Tables 140 and 141.

Table 128

Proximate Composition of Buckwheat and Its Mill Products

Product	Moisture, %	Protein, %	Fat, %	Fiber, %	N-free extract, %	Ash, %
Entire grain:						
Japanese variety	10.0	10.7	2.4	11.4	63.8	1.7
Silverhull variety	10.0	11.8	2.4	9.9	64.1	1.8
Flour:						
Very light color	12.6	4.7	0.5	0.4	81.2	0.6
Light color	13.1	7.2	1.3	0.6	76.6	1.2
Medium	12.1	9.8	1.8	1.0	73.9	1.4
Dark	9.7	11.9	2.8	1.0	72.9	1.7
Very dark	13.9	15.9	3.3	1.2	63.5	2.2
Groats:						
Japanese	11.1	12.6	2.8	0.8	71.0	1.7
Silverhull	10.2	14.1	2.9	0.9	70.0	1.9
Middlings (feed), containing 6.4% hulls	10.7	33.2	8.6	6.6	35.7	5.2
Middlings (feed), containing 18.3% hulls	10.1	26.0	6.5	16.5	36.4	4.5
Feed, high grade	9.5	17.3	4.5	19.3	46.1	3.3
Feed, low grade, containing 58.6% hulls	.4	11.9	2.8	36.5	38.8	2.6
Hulls:						
Japanese variety	6.4	2.9	0.8	49.4	38.5	2.0
Silverhull variety	6.4	3.1	0.5	52.1	36.1	1.8

TABLE 129

AVERAGE PROXIMATE COMPOSITION OF CEREALS AND THEIR BY-PRODUCTS USED FOR FEED[a, b]

Product	Moisture, %	Protein, %	Fat, %	Fiber, %	N-free extract, %	Ash, %
Barley, lightweight	10.2	12.3	2.3	8.5	63.7	3.0
Barley, hull-less or bald	9.8	11.6	2.0	2.4	72.1	2.1
Barley, bran, nearly all hulls	8.1	5.9	1.3	26.4	51.8	6.4
Barley feed, low grade	9.9	12.3	3.5	14.7	56.2	5.3
Barley feed, high grade	8.0	13.2	3.5	8.4	61.0	4.0
Barley malt	6.6	12.7	2.1	5.4	70.9	2.3
Barley screenings	11.4	11.5	2.8	9.5	60.6	4.2
Brewers' grains, dried, 25% protein or over	7.4	26.6	6.8	14.6	41.0	3.6
Brewers' grains, dried, 23–25% protein	6.1	23.8	6.5	14.9	44.9	3.8
Brewers' grains, dried, below 23% protein	7.0	21.6	6.2	16.1	45.2	3.9
Brewers' grains, dried, from California barley	8.9	20.0	5.7	18.1	43.6	3.7
Buckwheat feed, good grade	10.9	18.6	5.0	18.3	43.1	4.1
Buckwheat feed, low grade	11.7	13.3	3.4	28.6	39.8	3.2
Buckwheat flour	12.5	8 6	1.7	0.7	75.3	1.2
Buckwheat kernels, without hulls	10.6	11.2	2.4	0.7	73.6	1.5
Buckwheat middlings	11.3	29.7	7.3	7.4	39.4	4.9
Corn bran	9.9	9.8	6.4	9.8	61.8	2.3
Corn feed meal	10.8	10.0	4.4	3.0	70.1	1.7
Corn flour	12.2	8.6	4.0	0.7	73.3	1.2
Corn germ meal	7.0	19.8	7.8	8.9	53.2	3.3
Corn gluten feed, all analyses	9.5	26.4	2.5	7.1	48.4	6.1
Corn gluten feed, 27% protein or over	9.4	28.0	2.5	6 8	47 4	5.9
Corn gluten feed, 24–27% protein	9.4	25.8	2.5	7.3	48.9	6.1
Corn gluten feed, below 24% protein	10.2	22.9	2.5	7.5	49.8	7.1
Corn gluten meal, all analyses	8.5	42.9	2.3	2.5	42.0	1.8
Corn gluten meal, 43% protein or over	8.5	44.4	1.9	2.3	41.2	1.7
Corn gluten meal, below 43% protein	8.5	41.8	2.5	2.6	42.7	1.9
Corn meal, degermed and bolted	10.2	8.8	2.5	1.1	76.5	0.9
Corn oil meal, old process	8.2	22.9	7.5	10.0	49.1	2.3
Corn oil meal, solvent process	8.8	23.8	1.8	11.3	52.3	2.4
Distillers' corn grains, dried	6.4	30.6	10.6	10.8	38.7	2.9
Distillers' rye grains, dried	6.0	18.1	6.9	17.0	48.8	3.2
Hominy, pearled	10.6	7.8	3.3	1.0	76.1	1.2
Hominy, feed	9.1	11.0	6.9	4.8	65.5	2.7
Hominy, feed, low in fat	9.8	10.2	4.5	4.2	69.1	2.2
Oats, lightweight	8.7	12.3	4.7	15.4	54.4	4.5
Oats, mill, or low grade	8.5	11.6	5.3	12.0	58.6	4.0
Oat clippings	7.8	8.8	2.3	25.3	44.9	10.9
Oat flour	8.0	15.0	5.8	2.6	66.7	1.9
Oat kernels, without hulls	8.3	16.2	6.4	1.9	65.3	1 9
Oat meal, feeding, or rolled oats	8.5	16.3	5.9	2.8	64.1	2.4
Oat middlings	7.9	15.3	5.5	9.9	57.3	4.1
Oat mill feed, usual grade	6.3	5.5	2.1	27.6	52.3	6.2
Rice, brewers'	12.0	7.4	0.6	0.8	78.4	0.8
Rice, brown	9.8	8.9	2.0	1.0	77.2	1.1
Rice, polished	12.2	7.4	0.4	0.4	79.1	0.5

TABLE 129 (*Continued*)

AVERAGE PROXIMATE COMPOSITION OF CEREALS AND THEIR BY-PRODUCTS USED FOR FEED[a, b]

Product	Moisture, %	Protein, %	Fat, %	Fiber, %	N-free extract, %	Ash, %
Rice, bran	8.9	12.8	13.4	13.0	41.1	10.8
Rice, bran, low grade	9.9	10.0	7.9	20.2	36.8	15.2
Rice polish	9.5	12.7	11.5	3.0	57.2	6.1
Rye feed	9.6	16.0	3.3	4.6	62.8	3.7
Rye flour	11.6	8.3	1.3	0.6	77.3	0.9
Rye flour middlings	10.1	16.9	3.6	4.2	60.2	3.2
Rye middlings	9.8	16.5	3.4	5.0	61.5	3.8
Rye middlings and screenings	9.8	16.6	3.8	5.9	59.7	4.2
Wheat bran, chiefly hard red spring	9.1	15.9	5.5	9.6	53.9	6.0
Wheat bran, winter	9.1	15.7	4.2	9.1	55.1	6.4
Wheat, brown shorts	9.9	17.8	4.7	6.2	57.0	4.4
Wheat flour, graham	11.8	13.8	2.1	1.9	68.9	1.5
Wheat flour, low grade	11.2	15.1	2.5	0.8	69.1	1.3
Wheat flour, white	12.5	11.5	1.6	0.4	73.4	0.6
Wheat flour, middlings	10.4	17.0	4.9	4.4	59.9	3.4
Wheat germ meal	8.9	28.5	10.7	2.5	44.9	4.5
Wheat, gray shorts	9.9	17.9	4.5	5.6	57.8	4.1
Wheat, mixed feed	9.4	16.8	4.9	7.2	56.9	4.8
Wheat, red dog	10.8	16.9	4.0	2.4	63.3	2.6
Wheat, screenings	9.6	13.9	4.7	9.0	58.2	4.6
Wheat, standard middlings, all analyses	10.0	17.4	5.5	6.8	56.1	4.2
Wheat, standard middlings, chiefly hard red spring wheat	10.2	17.6	5.6	6.2	56.4	4.0
Wheat, white shorts	10.3	16.1	3.1	2.9	65.1	2.5

[a] Taken by special permission of the Morrison Publishing Company, Ithaca, New York, from the extensive Appendix Tables of *Feeds and Feeding*, 20th Edition, by F. B. Morrison.

[b] See also Table 119.

taining various combinations of buckwheat, wheat, rice, and corn flours with skim milk solids, salt, and leavening agents.

2. Chemical Composition

Typical analyses of the grain and its mill products, as given by Coe,[59] are shown in Table 128. The light-colored or short extraction flour is very low in protein content; as the extraction is lengthened, more and more of the embryo and outer parts of the kernel are included in the flour and hence the protein, fat, fiber, and ash increase.

Average proximate analysis and the vitamin and mineral composition of buckwheat flours are recorded in Tables 126 and 127; the proximate analyses of feed by-products are given in Table 129.

V. CORN OR MAIZE

1. Utilization

The utilization of the tremendous corn crop of the United States is summarized by the data in Table 130. Of the average annual production of some 2.25 billion bu.,

---

[59] M. R. Coe, U. S. Dept. Agr., *Circ.* 90 (1931).

TABLE 130

AVERAGE ANNUAL DISAPPEARANCE OF CORN FOR SPECIFIC PURPOSES FOR THE TEN CROP YEARS 1930–1931 TO 1939–1940, INCLUSIVE[a]

Disposition	Quantity, bu. 1000 bu.	Disposition, %	
		Total	Food and industrial uses only
Food and industrial uses:			
Dry process products	41,353	..	29.4
Alcoholic beverages[b]	22,156	..	15.8
Wet process products	69,947	..	49.7
Breakfast foods	7,153	..	5.1
Total	140,609	6.2	100.0
Other uses:			
Feed, seed, farm families, export, and other uses	2,110,996	93.8	...
Total disappearance	2,251,605	100.0	...

[a] Compiled from *Agricultural Statistics*, 1941.

[b] The average value includes three years when prohibition was in force; in these years the utilization of corn for production of alcoholic beverages was only about $2^1/_2$ to 7 million bu.

only about 141,000,000 bu., or 6% of the total crop, is processed for food and industrial uses. Approximately 50% of the corn (70,000,000 bushels) which is processed is used by the wet-milling industry for the production of cornstarch and other carbohydrates derived from it, corn oil, and various feed by-products; products made by the wet-milling industry from corn and their uses are listed in Table 131. Of the remainder, about 34.5% is utilized in the preparation of various food products and the balance for making alcoholic beverages.

Corn is prepared in a number of ways for use as human food: parched to be eaten whole, ground to varying degrees of fineness to make hominy, corn meal, and corn flour, treated with alkali to remove the pericarp and germ to make lye hominy, and converted into a variety of breakfast foods by special processes. Most of the corn consumed as human food is in the form of corn meal. In 1939, the per capita consumption of corn meal was 23.4 lbs. and an additional 8 lbs. was consumed as breakfast foods, hominy, and canned corn. Yellow corn products are preferred in the northern States; white corn products in the southern States and Rhode Island; and blue, black, and red corn products in the Southwest where the Spanish influence is dominant.

2. Chemical Composition of Kernel Structures

In describing the structure of the corn kernel (Fig. 52, page 601), reference was made to the layer of horny gluten underneath the hull, and to the existence of horny starch and floury starch. Hopkins, Smith, and East[60] have dissected low, medium, and high protein dent corn kernels into various parts and determined their relative amounts and chemical composition; their average results are given in Table 132. The embryo is

---

[60] C. G. Hopkins, L. H. Smith, and E. M. East, Ill. Agr. Expt. Sta., *Bull.* 87 (1903).

Table 131

List of Products Made from the Corn Kernel[a]

STARCHES	DEXTRINS	SIRUPS	SUGARS	CORN OIL	FEEDS	MISCELLANEOUS
Alkaline Bakers' Binder Boraxed Brewers' Grits Chlorinated Confectioners' Crystal Gelatinized Lump Noncongealing Pearl Powdered Soluble Thick Boiling Thin Boiling	British Gum Canary Dextrin Founders' Mixture Special Dextrins Special Gums White Dextrin	Brewers' Corn Sirup High, Low and Medium Conversion Corn Sirups Mixing Corn Sirup Special Tanners' Corn Sirup	Corn Sugar Eighty Corn Sugar Seventy Corn Sirup Solids Dextrose, Anhydrous Dextrose, Hydrate Refined Bread Sugar	Black Grease Corn Oil, Crude Corn Oil, Refined Corn Oil, Solidified Soap Stock Solid Soap	Corn Bran Corn Gluten Feed Corn Gluten Meal Corn Oil Cake Corn Oil Cake Meal	Calcium Lactate Corn Sugar Molasses (Hydrol) Lactic Acid Pudding Powders Sodium Lactate Yeast Zein
			—and Their Uses			
Asbestos Baking Baking Powders Brewing Confectionery Cosmetics Explosives Household Laundry Paper Pastes Pharmaceuticals Sizings Soaps Textiles	Carpets Colors Fireworks Glues Gums Leather Mucilage Paper Pastes Sizings Textiles Twine Window Shades	Baking Brewing Canned Fruits Carbonated Beverages Confectionery Food Sauces Fruit Butters Fruit Juices Ice Cream Infant Feeding Jams, Preserves Jellies Marmalades Mayonnaise Meat Products Pharmaceuticals Pickles Relishes Table Sirups Tobacco	Baking Brewing Canned Fruits Caramel Confectionery Dietetics Fiber Silk (Rayon) Fruit Butters Fruit Juices Ice Cream Jams, Preserves Jellies Marmalades Meat Products Pharmaceuticals Pickles Sauces Soft Drinks Sugar Color Tanning Vinegar	Commercial Baking Pharmaceuticals Salad and Cooking Oil Soaps Textiles	Beef Cattle Dairy Cattle Poultry Sheep	Dietary Experimental Uses Foods Medical Plastics Tanning

[a] Reproduced from *Corn in Industry* with permission of the Corn Industries Research Foundation, New York.

rich in protein and contains about 85% of the total fat and 75% of the total ash present in the kernel. The horny gluten is very high in protein content and it will be noted also that the horny starch contains about 2% more protein than the soft or floury portion of the endosperm. In the high and low protein strains, the differences in the protein content of the kernel were found to be due almost entirely to a higher protein content in the endosperm; similarly, in high oil strains, it was the embryo which showed the greatest differences in oil content.

TABLE 132

AVERAGE DISTRIBUTION AND CHEMICAL COMPOSITION OF VARIOUS PARTS OF THE CORN KERNEL[a]

Part	Proportion of kernel	Chemical composition (dry matter basis), %			
		Protein	Fat	Carbo-hydrates	Ash
Tip cap[b]	1.4	7.0	1.6	90.0	1.4
Hull	5.8	4.2	0.9	94.0	0.9
Horny gluten[c]	11.1	21.8	5.0	71.4	1.5
Horny starch[d]	43.0	9.9	0.2	89.7	0.2
Crown starch	17.4	7.8	0.3	91.6	0.3
Tip starch	10.2	6.9	0.6	92.1	0.4
Embryo	11.0	19.7	34.9	35.2	10.0
Entire kernel	..	11.0	4.6	82.9	1.5

[a] Computed from data of Hopkins, Smith, and East[60] for low, medium, and high protein corn.
[b] Loose, chaffy tissue at base of kernel.
[c] Outer endosperm including aleurone cells.
[d] Inner horny endosperm.

3. Definitions and Standards for Dry Milled Corn

Standards for corn products have not yet been promulgated under the Federal Food, Drug, and Cosmetic Act of 1938 but certain rather well-defined standards have been established by custom in the corn trade. These are given below.[61]

Pearled Hominy.—Made from degermed, hulled, clean, sound corn; screened through a No. $3^1/_2$ wire screen and over a No. 6 wire screen; contains not more than 14% moisture and 1% fat.

Coarse Table Grits.—Made from pearled hominy or degermed, hulled, clean, sound corn; ground and screened through a No. 10 wire and over a No. 14 wire; contain not more than 14 % moisture and 1% fat.

Medium Table Grits.—Made from pearled hominy or degermed, hulled, clean, sound corn; ground and screened through a No. 14 wire and over a No. 24 wire; contain not more than 14% moisture and 1% fat.

Fine Table Grits, Granulated Corn Meal.—Made from pearled hominy or degermed, hulled, clean, sound corn; ground and screened through a No. 24 wire and over a No. 46 wire; contain not more than 14% moisture and 1.5% fat.

Cream Corn Meal or Pearl Corn Meal.—Made from pearled hominy or grits; screened through a No. 24 wire and dusted over a No. 72 grits gauze, or equivalent wire cloth; contains not more than 14% moisture and 2.5% fat. (This product is frequently called "corn cones.")

---

[61] Courtesy of American Corn Millers Federation, Chicago, Ill.

Standard Corn Meal.—Made from clean, sound corn with approximately 10% of the feed removed, and approximately 45% of cream meal or grits extracted; contains not more than 13.5% moisture.

Bolted Corn Meal.—Made from clean, sound corn with approximately 5% of the bran and germ removed; contains not more than 14% moisture.

Old-Fashioned Rock or Water-Ground Corn Meal.—Made from clean, sound corn ground to a soft meal; contains not more than 14% moisture.

Corn Flour.—Made from clean sound corn, hominy, grits, or cream meal; will pass through a No. 72 grits gauze or equivalent wire cloth; contains not more than 13.5% moisture and 2.5% fat.

The Association of American Feed Control Officials has approved the following definitions for corn by-product feeds produced in the dry-milling process.

Corn Chop, Ground Corn, or Cracked Corn is the entire product made by grinding, cutting, or chopping the grains of sound Indian corn, and may be fine, medium, or coarse, and must not contain more than 4% of foreign material.

Screened Corn Chop, Screened Ground Corn, or Screened Cracked Corn is the coarse portion of corn chop, ground corn, or cracked corn from which most of the fine particles have been removed, and must not contain more than 4% of foreign material.

Corn Meal (Feeding) is finely ground, unbolted corn.

Corn Bran is the outer coating of the corn kernel, with little or none of the starchy part or germ.

Corn Feed Meal is the fine siftings obtained in the manufacture of screened corn chop, screened ground corn, or screened cracked corn, with or without its aspiration products added.

Corn Grits, Hominy Grits is the product consisting of the fine or medium-sized, hard, flinty portions of sound Indian corn containing little or none of the bran or germ.

Corn Screenings is the product consisting of the small, light grains of corn, parts of grains of corn or other cereals, and other materials having feeding value, separately or together, obtained by screening shelled corn, excluding sand, dirt, and other similar inert materials.

Ear Corn Chops is corn and cob chopped, without the husk, with no greater proportion of cob than occurs in the ear corn in its natural state.

Hominy Feed is a mixture of corn bran, corn germ, and a part of the starchy portion of either white or yellow corn kernels or mixture thereof as produced in the manufacture of pearl hominy, hominy grits, or table meal, and shall contain not less than 5% of crude fat. If prefixed with the words "white" or "yellow," the product must correspond thereto.

4. Chemical Composition of Corn Products from Dry Milling

The chemical composition of corn meals is given in Tables 126 and 127 while that of the feed by-products is shown in Table 129. The composition of old-process or undegerminated corn meal is practically the same as that of the corn from which it is milled. In the instance of the more highly refined products such as hominy and cream meal, the bran and germ have been more or less completely removed so that the protein, vitamin, and mineral contents are considerably lower than those of the entire grain; these products are analogous to wheat "farina" and "patent" wheat flour in purity, whereas the less highly refined standard meal corresponds to "clear" wheat flour.[62] Yellow and white corn meals of equivalent refinement are similar in composition, except that the former contains from 3.5 to 5.0 I. U. of vitamin A per g. The susceptibility of corn meal to attack by insects and to deterioration from spoilage is greatly lessened by removal of the germ and by drying to a relatively low moisture content. The relation between high moisture content and the rate of development of acidity in corn and its products has long been known[62-64] and recently it has been shown that the fat acidity, in particular, is a reliable index of the relative soundness of corn.[65-67]

---

[62] A. L. Winton, W. C. Burnet, and J. H. Bornmann, U. S. Dept. Agr., *Bull.* **215** (1915).

5. Feed By-Products—Definitions and Chemical Composition of Corn Products from Wet Milling

The following definitions have been approved by the Association of American Feed Control Officials for by-product feeds resulting from the corn wet-milling process.

Corn Gluten Feed is that part of the commercial shelled corn that remains after the extraction of the larger part of the starch and germ, and the separation of the bran by the process employed in the wet-milling manufacture of cornstarch or sirup. It may or it may not contain one or more of the following: corn solubles, corn oil meal.

Corn Gluten Meal is that part of the commercial shelled corn that remains after the extraction of the larger part of the starch and germ, and the separation of the bran by the process employed in the wet-milling manufacture of cornstarch or sirup. It may or it may not contain one or more of the following: corn solubles, corn oil meal.

Corn Oil Cake consists of the corn germ from which part of the oil has been pressed, and is the product obtained in the wet-milling process of manufacture of cornstarch, corn sirup, and other corn products.

The average chemical composition of the various feed by-products is given in Table 129.

VI. OATS

1. Utilization

From 80 to 85% of the oats grown are fed to livestock. According to the U. S. Census Report, some 529,673,000 lbs. of rolled oats and oat meal were manufactured in 1939 with a monetary value of approximately $25,000,000. Oat milling is the leading cereal industry in the production of breakfast cereals. Oat flour has antioxidant properties; that is, it tends to retard the development of rancidity and is finding an increased use in food mixtures where more potent chemical antioxidants cannot be employed because of their toxicity.[68] Oat meal and oat flour are also used in the manufacture of special soaps and certain cosmetics used for cleansing and beautifying purposes.

2. Chemical Composition of Rolled Oats

The data in Tables 126 and 127 show that rolled oats and oat meal are high in protein, fat, and energy value and are the richest sources of calcium, phosphorus, iron,

---

[63] J. Schindler, *Z. landw. Versuchsw. Deut.-Oesterr.*, 12, 721 (1909).
[64] J. S. McHargue, *Ind. Eng. Chem.*, 12, 257 (1920).
[65] L. Zeleny, and D. A. Coleman, *Cereal Chem.*, 15, 580 (1938).
[66] L. Zeleny, and D. A. Coleman, U. S. Dept. Agr., *Tech. Bull.* 644 (1939).
[67] L. Zeleny, *Cereal Chem.*, 17, 29 (1940).
[68] F. N. Peters, and S. Musher, *Ind. Eng. Chem.*, 29, 146 (1937).

and thiamine, as compared with other refined cereals. It is possible to introduce a small amount of vitamin D (possibly up to 4 or 5 I. U. per ounce) into rolled oats by exposing them to the action of high-intensity ultraviolet light for a period of a few seconds; higher potencies can be obtained, but these are usually accompanied by the development of an off-flavor. Any standard source of ultraviolet irradiation may be employed but the most widely used source is a cold quartz lamp.[69] The high nutritive value of food products manufactured from oat groats is associated with the retention of the bran and the germ in the milling process.

3. Definitions and Composition of Oat By-Products

Oats Hulls is the product consisting of the outer covering of the oat.

Oat Middlings is the product consisting of the floury portions of the oat groat obtained in the milling of rolled oats.

Oat Shorts is the product consisting of the covering of the oat grain lying immediately inside the hull, being a fuzzy material carrying with it considerable portions of the fine floury part of the groat obtained in the milling of rolled oats.

Clipped Oat By-Product is the by-product obtained in the manufacture of clipped oats. It may contain the light, chaffy material broken from the end of the hulls, empty hulls, light, immature oats, and dust. It must not contain an excessive amount of oat hulls.

Oat Chop, Ground Oats, Pulverized Oats, Crushed Oats, or **Crimped Oats** consists of the entire product made by grinding, chopping, cutting, crushing, or crimping whole oats.

Oat Groats are the kernels produced from cleaned and dried oats in the process of manufacturing oatmeal.

Hulled Oats, Undried Oat Groats are the kernels produced from the undried grain in the process of hulling oats.

Feeding Oatmeal is a product obtained in the manufacture of rolled oat groats or rolled oats and consists of broken rolled oat groats, oat groat chips, and floury portions of the oat groats, with only such quantity of finely ground oat hulls as is unavoidable in the usual process of commercial milling. It must not contain more than 4% of crude fiber.

Oat Millfeed (Oat Hulls, Oat Shorts, and Oat Middlings) is the entire by-product produced in the manufacture of oat groats and consists of oat hulls, oat shorts, and oat middlings. If used in a mixed feed, it shall be called Oat Millfeed (Oat Hulls, Oat Shorts, and Oat Middlings).

Cut Oat Groats, Cracked Oat Groats, or **Ground Oat Groats** is the product produced by cutting, cracking, or grinding oat groats.

The average proximate composition of oats and certain oat products used in livestock feeding is given in Table 129. Oat hulls are not included in the compilation since they have relatively little feeding value, being poor in food nutrients and of low digestibility. They contain about 4% protein, 1.5% fat, 29% fiber, 52% nitrogen-free extract, and 6% ash. Oat hulls are rich in pentosans and the problem of their economic utilization was solved by conversion of the pentosans to furfural by destructive distillation with hot dilute acid. Furfural, liquid of high boiling point, is an important industrial chemical with a wide variety of uses as a solvent, as a bactericide, fungicide, and in the manufacture of resins; it also serves as the starting material for other furans of industrial importance.[70]

---

[69] F. L. Gunderson, *Food Industries*, **13**, No. 6, 48 (1941).

[70] F. N. Peters, *Ind. Eng. Chem.*, **28**, 755 (1936).

VII. RICE

CULTIVATED RICE

1. Utilization

The average annual rice production of approximately 40,000,000 bushels in the States of Louisiana, Texas, Arkansas, and California, exceeds domestic requirements and usually about one-fourth of the crop is exported. The annual U. S. production of milled rice averaged approximately 1269 million lbs. for the 10-year period 1930–1939, inclusive. The per capita consumption of milled rice in the continental United States is only about 5.5 lbs. per capita, as compared with over 200 lbs. in India, French Indo-China, Java, and the Philippines and from 300 to 400 lbs. in Japan.

2. Kinds of Rice

Rice is a covered cereal; in the threshed grain, or rough rice, the kernel (or caryopsis) is enclosed in a tough siliceous hull. Photographs of the three common types, long-, medium-, and short-grain, are reproduced in Fig. 55. When the hull is removed, the caryopsis, comprising the pericarp (outer bran) and the seed proper (inner bran, endosperm, and germ), is obtained. This form of rice is known as brown rice or sometimes

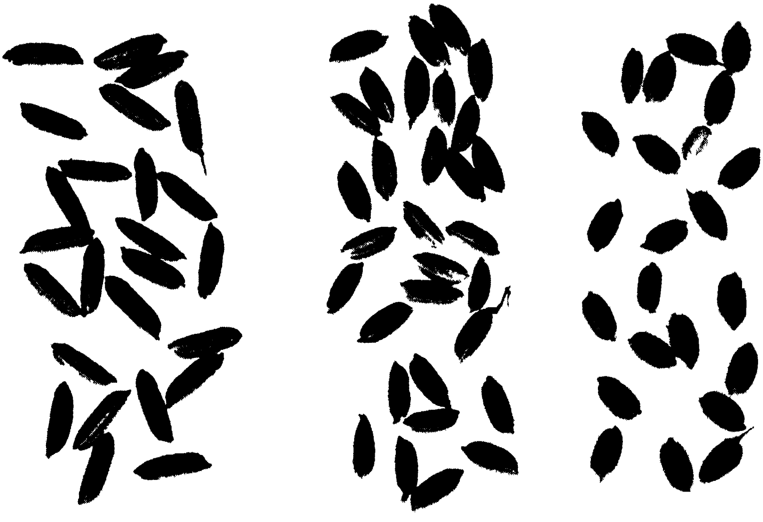
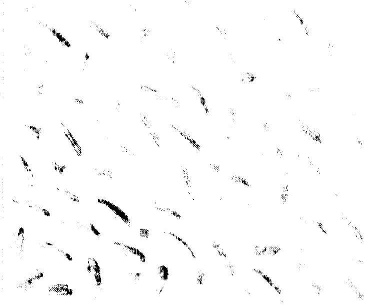
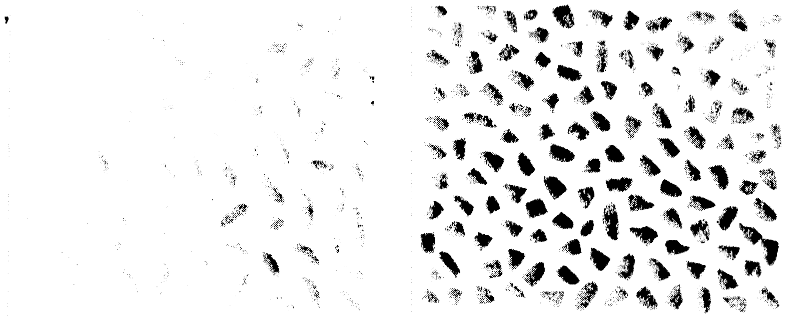


Fig 55 —Rough rice grains illustrating the three common types produced in the United States Left, long-grain type (Fortuna variety), center, medium-grain type (Blue Rose variety); and right, short-grain type (Japan). Threshed grain of the long types is 9 0–9 8 mm. long and 2.4–3.4 mm. thick; medium-grain varieties are 8 0–8.7 mm long and 3 2–3.4 mm. thick; short-grain varieties average about 7 2 mm in length and 3 7 mm. in thickness. (Courtesy of W. D. Smith, Bur. of Agr. Economics, U. S Dept Agr).

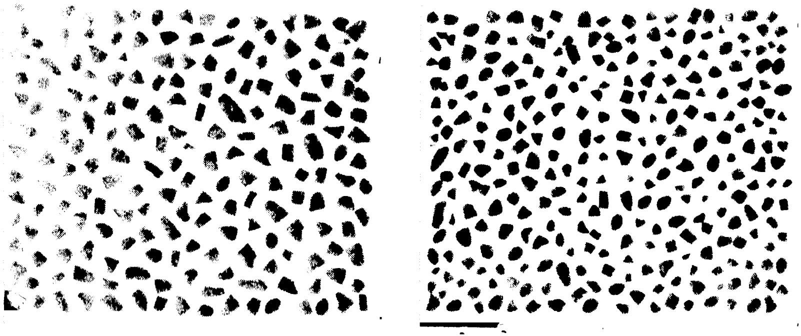
as unpolished rice. When subjected to further milling processes, the bran (including the aleurone layer) and germ are removed and the purified starchy endosperms are



Brown Rice



Head rice Second head rice



Screenings rice Brewers' rice

Fig. 56.—Photographs of brown rice and various classes of milled or polished rice. (Courtesy of W. D. Smith, Bur. of Agr. Economics, U. S. Dept. Agr.)

marketed as white rice, or polished rice which is classified according to size as head rice (whole endosperm), and various classes of broken rice, known as second head, screenings, and brewers' rice, in order of decreasing size. Photographs of brown rice and the various classes of white rice are shown in Fig. 56.

Official standards for milled rice provide for 14 classes with 6 grades (extra fancy, fancy, extra choice, choice, medium, and sample grade). Head rice is sorted into 11 different classes on the basis of variety; the remaining classes provide for second head, screenings, and brewers' milled rice. The principal factors considered in grading milled rice are: cereal grains, weed seeds, damaged kernels, red rice, chalky kernels, broken kernels, and moisture. Two kinds of damaged kernels are recognized: namely, "heat-damaged" kernels (amber or mahogany in color) and "damage other than heat." The latter class largely comprises kernels which have discolored spots as a result of fungal growth and are known as "pecky kernels." Red rice is objectionable because of the effect upon general appearance. Chalky kernels are more easily broken in milling, and generally cook more rapidly than the flinty kernels.

3. Chemical Composition of Rice and Its Mill Products

The proximate composition of rough rice does not appear to vary greatly, as judged from published analyses of different types and varieties grown in various countries which have been assembled by Winton and Winton;[71] for example, the range in protein content is in the order of only 2 to 3%. This is, perhaps, a consequence of the fact that, during the greater part of the growing period, the land is submerged and variations in the moisture supply, which have such a pronounced effect upon the composition of other cereals, do not come into full play.

TABLE 133

AVERAGE CHEMICAL COMPOSITION OF CULTIVATED RICE AND ITS BY-PRODUCTS[a]

Product	Proximate composition, %						Sugars, %		Pentosans, %
	Moisture	Protein	Fat	Fiber	N-free extract	Ash	Reducing	Disaccharide	
Rice:									
Rough rice	11.7	8.1	1.8	8.9	64.5	5.0	...	...	..
Brown rice	12.2	9.1	2.0	1.1	74.5	1.1	0.1	0.8	2.1
Head rice	12.6	9.0	0.5	0.4	77.0	0.5	0.1	0.2	1.8
Second head rice	12.4	8.7	0.4	0.4	77.5	0.6	0.1	0.2	1.9
Screenings rice	11.4	8.4	0.5	0.4	78.7	0.6	Trace	0.3	1.8
Brewers' rice	11.8	8.9	1.0	0.6	77.0	0.7	0.2	0.2	1.7
By-products:									
Stone bran	9.7	9.8	7.7	20.9	36.7	15.2	0.8	1.2	13.9
Huller bran	9.8	15.0	16.9	7.9	43.0	7.4	1.2	4.5	7.8
Cone bran	9.8	15.4	16.0	5.7	46.0	7.1	1.6	2.2	5.8
Polish	9.9	12.9	9.1	2.1	61.8	4.2	1.3	2.3	3.5
Hulls	8.5	3.6	0.9	39.0	29.4	18.6	0.2	0.4	18.1

[a] Compiled from data of G. S Fraps, Texas Agr. Expt. Sta., *Bull.* 191 (1916).

Many comparative analyses of American rice and its mill products have been published, but the average data given in Table 133, obtained by Fraps[72] in a comprehensive survey of Texas rice mills, will serve to illustrate the relative proximate composition of the various mill products. The hulls are low in protein and fat and high in crude fiber and ash, so that their removal, in the production of brown rice, results in a rise in the protein, fat, and nitrogen-free extract figures and a decrease in crude fiber and ash.

---

[71] A. L. Winton and K. Winton, *The Structure and Composition of Foods.* Vol. I, Wiley, New York, 1932.

In the conversion of brown rice to white or polished rice, about 10% of the protein, 85% of the fat, and 70% of the minerals are removed. The "bran" from the shelling stones consists quite largely of pulverized hulls and is much lower in protein and fat and higher in fiber and ash content than the "huller" and "cone" bran which is composed of the true bran and germ. Commercial rice bran, a valuable concentrate for livestock, consists of a mixture of the stone-huller and cone brans. The rice polish, obtained in the final scouring process carried out in the "brush," is composed of the very thin, inner seed coat layers together with appreciable quantities of endosperm material. The pentosans are found chiefly in the hulls and, to a lesser extent, in the bran. Wise and Boomel[73] found that about 60% of the pentosans was removed with the hulls and 33% of the remainder was lost during the subsequent scouring processes.

Recent data on the B complex vitamins in U. S. rice and its mill products are summarized in Table 134. There is little difference between the vitamin contents of rough and brown rice, but as the latter progresses through the successive stages of milling, the vitamin values decrease. The greatest decrease occurs at the first break. Head rice, the principal form of white rice used for human consumption, contains only about 20% as much thiamine, 50% as much riboflavin, and 34% as much nicotinic acid (niacin), as brown rice.

The reduction in thiamine attributed to milling is particularly significant; and in rice-eating countries, such as Java, the Philippines, and Japan, where rice is the most important ingredient of the diet, the continuous and exclusive use of polished rice results in beriberi, a deficiency disease which arises from an abnormally low thiamine intake.

Because of these losses, there has been much interest recently in the development of practical methods for retaining more of the B vitamins in the milled rice. This problem has been approached in two ways, namely, by removing less of the bran layers and germ in milling, and by processing the rice prior to milling in such a way as to cause diffusion of the vitamins and other water-soluble nutrients present in the outer portions of the grain into the endosperm.

Kik and associates[74-74e] have been particularly active in investigating the value of the various methods which have recently been introduced. The first of these methods involves the production of so-called under-milled rice. The term "unpolished rice" has recently been adopted by the rice industry for this type of product. Unpolished rice is rice from which the outer bran layers and part of the germ have been removed but on which the inner bran layers, which are normally removed in the polishing process, are retained. Since the greatest decrease in B vitamins occurs at the first break, it seems clear that only limited improvement in vitamin values can be effected by decreasing the degree of refinement brought about in subsequent stages of the milling process. Limited trials of the Earle peeling process, first used with wheat (see Vol. II, Chapter XV), indicate that high vitamin retention is possible by the production of peeled rice.

---

[72] G. S. Fraps, Texas Agr. Expt. Sta., *Bull.* **191** (1916).

[73] F. B. Wise, and A. W. Boomel, U. S. Dept. Agr., *Bull.* **330** (1916).

[74] M. C. Kik, *Cereal Chem.*, **20**, 103 (1943).

[74a] M. C. Kik, and F. B. Van Landingham, *Cereal Chem.*, **20**, 563 (1943).

[74b] M. C. Kik, and F. B. Van Landingham, *Cereal Chem.*, **20**, 569 (1943).

[74c] M. C. Kik, *Rice*, **46**, No. 4, 21 (April, 1943).

[74d] M. C. Kik, *Rice*, **46**, No. 6, 14 (June, 1943).

[74e] M. C. Kik, and F. B. Van Landingham, *Cereal Chem.*, **21**, in press.

TABLE 134

B COMPLEX VITAMINS IN RICE AND ITS MILL PRODUCTS (DRY MATTER BASIS)

Product	Data of Kik and Associates[a] Averages for seven mill lots, γ/g.			Data of Williams, Knox, and Fieger[b] Averages for composite samples of three varieties, γ/g.			
	Thiamine	Riboflavin	Niacin	Thiamine	Niacin	Pantothenic acid	Pyridoxine
Rough rice	2.93	0.67	49.2	..	..	..	..
From milling process:							
Brown rice	3.02	0.53	55.1	4.2	47.2	17.0	10.3
First break huller rice	0.91	0.27	23.7	1.7	25.8	9.1	6.9
Second break huller rice	0.83	0.27	22.4	1.2	21.7	7.7	5.1
Pearling cone rice	0.82	0.29	22 6	..	..	..	..
Brush rice	0.64	0.25	20.4	0.8	12.3	7.6	5.1
Finished white rice:							
Head rice	0.60	0 26	18.5	0.8	12.7	6.4	4.5
Second head rice	0.50	0 25	17.5	..	..	..	..
Screenings	0.79	0.34	23.8	..	..	..	..
Brewers' rice	1.41	0.36	36.1	..	..	..	..
By-products:							
Hulls	1.11	0.76	18.6	..	..	..	..
First break bran	27.33	2.68	332.0	27.9	408.6	71.3	32.1
Second break bran	17.86	1.77	286.3				
Pearling cone polish	22.88	1.62	290.3	23.3	384.7	92.5	30.8
Brush polish	20.95	1.34	368.0				

[a] Thiamine data from M. C. Kik, *Cereal Chem* , 20, 103 (1943); riboflavin data from M. C. Kik and F. B. Van Landingham, *ibid.*, **20**, 563 (1943); niacin data from M. C. Kik and F. B. Van Landingham, *ibid.*, **21** (in press).

[b] R. Williams, W. C. Knox, and E. A. Fieger, *Cereal Chem.*, **20**, 560 (1943).

The method of increasing vitamin retention by processing the rough rice prior to milling involves parboiling or some modification thereof. In its simplest form, parboiling consists of soaking the rough rice in water, draining off the surplus water, then steaming, and finally drying the rice. Malekized rice, a product produced in California, is made by milling rough rice after it has been subjected to a modified parboiling treatment consisting of soaking, steaming under pressure, and drying. In 1940, a patented process for the manufacture of so-called converted rice was developed in

England[74f] and has recently been put into commercial operation in Houston, Texas. This process involves, essentially, exposure of the cleaned rough rice to vacuum, followed by treatment with hot water under pressure, and then steam; the grain is then dried and milled.[74c]

Typical data for the B vitamin content of rice manufactured in various ways, as published by Kik,[74d] are as follows:

Product	Dry matter basis, γ/g.		
	Thiamine	Riboflavin	Niacin
Brown rice	3.50	0.60	60
Undermilled rice (unpolished)	1.22	0.32	26
Parboiled, milled rice	1.74	0.37	40
Peeled rice (Earle process)	3.00	0.42	50
Malekized rice (modified parboiling, milled)	2.00	0.40	44
Converted rice (modified parboiling, milled)	3.20	0.50	49

These data show that the parboiling treatment, particularly by the H. R. Conversion process, results in a marked increase in the retention of thiamine, riboflavin, and niacin in the milled rice. In a more recent study of the retention of these vitamins in 25 samples of converted rice produced commercially in Houston, Texas, Kik[74g] found that an average of 92.2% of the thiamine, 70.8% of the riboflavin, and 77.6% of the niacin originally present in the rough rice was retained. It has also been observed that this pretreatment greatly inceases the yield of head rice. By the old milling process, breakage with the long-grain varieties may approach 40%, whereas in the rice conversion method this is reduced to a maximum of about 10%. A recent compilation[74h] of the relative composition of brown and white rice in regard to nutritionally important mineral elements gives the following values:

Product	Calcium, %	Phosphorus, %	Iron, %	Copper, %
Brown rice	0.084	0.290	0.0020	0.00036
White rice	0.009	0.096	0.0009	0.00019

In addition to these reductions in mineral and vitamin content, it has been found that the biological value of the rice protein is decreased by the milling process. The endosperm protein is largely oryzenin, a glutelin, with little or no prolamin, albumin, or globulin, whereas the bran and germ contain these proteins. In a recent study of the relative nutritive values of the proteins of brown rice and its mill products, Kik[75] found that the proteins of brown and of polished rice had a higher digestibility but a lower biological value than those of rice bran and rice polishings; the biological values were as follows: brown rice, 72.7%; white polished rice, 66.6%; rice bran, 84.9%; rice polishings, 82.9%, as compared with 81.5% for casein when the various foods were fed at a 5% level. In a subsequent paper[76] he reported that the amino acids, cystine, methionine, and lysine, but not tryptophane, supplemented to a slight extent the proteins of brown and white rice in feeding experiments with rats. The addition of cystine to diets containing rice bran and rice polishings did not have a beneficial effect upon growth. Kik[77] has also determined cystine, tryptophane, lysine, arginine, and histidine in these rice products and found that white, brown, and polished rice have a rather low cystine and lysine content as compared with published data for wheat.

[74f] H. R. Rice Conversion (patent, 1940), Rice Conversion Ltd., 14 and 19 Leadenhall St., London, E. C. 3.

[74g] M. C. Kik, private communication, January, 1944.

[74h] *Nutritional Charts for Medical and Other Specialists.* 4th ed., H. J. Heinz Co., Pittsburgh, Pa., 1942.

[75] M. C. Kik, *Cereal Chem.*, **16**, 441 (1939).

The average proximate analyses of rice feed by-products are given in Table 129.

4. Utilization of Rice and Its Mill Products

Rough rice is used for seed and as a feed for livestock, but the presence of the harsh siliceous hulls renders it unsuitable for human consumption. Brown rice, despite its higher nutritive value, is in little demand as a food. It usually contains more impurities and defects than are present in completely milled rice. Unless stored under very favorable conditions, it has a tendency to become rancid and is more subject to insect infestation than the various forms of milled or white rice. The whole endosperms of milled rice, head rice, command the highest price and are used boiled, as a vegetable, and in the manufacture of such prepared breakfast foods as puffed rice and rice flakes. Broken rice (second head, screenings, and brewers' rice) is used as a human and livestock food, in making alcoholic beverages, and as a source of starch and flour. Rice flour is a common ingredient of griddle-cake mixtures.

Rice bran, consisting of bran and germ, with varying quantities of hulls, is the most nutritious of the by-products but, like rice polish, tends to become rancid on storage. Rice bran and rice polish are valuable concentrates for livestock; the polish is also used as a human food (thickening agent for gravies, sauces, and puddings, ingredient of sausages) and in the manufacture of buttons, rice oil, and soap.

Rice hulls, the principal by-product of rice milling, have little monetary value. They have very low feeding value and are used as fuel, packing, and insulating material, bedding for poultry and for the manufacture of α-cellulose. Hull ash is used as a bleaching agent, filler for bricks and concrete, in making soaps, polishing and cleansing agents, and as a source of sodium silicate.

WILD RICE (*Zizania aquatica*)

Wild rice is not closely related botanically to the cultivated species. It is a tall annual grass which grows partially submerged along the margins of lakes and is an important article of food of the Indian tribes of the Great Lakes and upper Mississippi Valley regions. The grain is borne on panicles from 15 to 24 in. long. Since it ripens from the top of the panicles downward and drops off when mature, the crop is harvested two or three times at intervals of about ten days during September. The Indians beat the grains off into the bottom of canoes forced among the plants. The grains are dried in the sun and then parched in iron pots or troughs over slow fires to loosen the hulls. The parched grains are then transferred to a large concrete pot in which a mocassin-clad Indian stands and shuffles or "dances" the rice to separate the hulls. The hulls are removed by Indian women and children by tossing the mixture of rice grains and chaff into the wind. The leading production is in Minnesota where an estimated annual crop of 350,000 to 500,000 lbs. is produced. It is highly prized as a table delicacy for serving with fowl and game. As the retail selling price is from 50 to 75 cents per pound, it is spoken of as the "world's costliest cereal."

---

[76] M. C. Kik, *Cereal Chem.*, **17**, 473 (1940)

[77] M. C. Kik, *Cereal Chem.*, **18**, 349 (1942).

The average proximate composition, calcium, and phosphorus content of parched wild rice processed by the Indians, as determined by Kennedy[78] together with the results of recent assays for vitamins of the B complex, by Nelson and Palmer[79] are as follows:

	%		%		γ/g.
Moisture	8.1	Ash	1.2	Thiamine	4.7
Protein	14.1	Nitrogen-free extract	74.4	Riboflavin	6.3
Fat	0.7	Calcium	0.018	Niacin	61
Crude fiber	1.5	Phosphorus	0.424	Pantothenic acid	10

In comparison with cultivated brown rice, parched wild rice is much higher in protein, somewhat lower in fat, apparently contains less calcium but more phosphorus, and is richer in thiamine and riboflavin. As compared with polished or white rice, the principal form in which cultivated rice is consumed, wild rice contains about twice as much protein, fat, and calcium, four times as much phosphorus, eight times as much thiamine, and twenty times as much riboflavin. Kennedy found the biological value of the proteins of parched wild rice to be superior to those of polished rice.

Wild rice is somewhat lower in thiamine content than the majority of the cereal grains, but is a much richer source of riboflavin (Tables 121 and 122).

VIII. RYE

1. Utilization

Rye is used as a feed for livestock, for the manufacture of whisky and the production of flour. During the 10-year period 1930–1939, an average of 19,797,000 bu. of rye (51% of the total production) was sold yearly from the farms on which it was produced, about one-third of which was ground or milled. In 1939, for example, 7,703,000 bu. of rye were ground, of which 85% was processed into flour (1,430,696 barrels) valued at $4,091,603 and the remainder into feeds. The annual consumption of rye flour in the United States has been 2.3 lbs. per capita in recent years.

---

[78] C. Kennedy, *J. Agr. Research*, **27**, 219 (1924).

[79] J. W. Nelson and L. S. Palmer, *Cereal Chem.*, **19**, 539 (1942).

2. Chemical Composition of Rye Mill Products

The average proximate composition, vitamin, and mineral content of rye flours are given in Tables 126 and 127, and the proximate composition of the mill offals in Table 129. As the grade of flour decreases, there is a marked increase in protein and ash content, as well as an increase in fiber. There is a considerable range in the ash content of the different grades of rye flour on the market as their purity varies rather widely, depending to a considerable extent upon the demand for rye feeds. When feed prices are high, it is more profitable for the miller to produce grades representing a shorter extraction and lower ash content than when there is a poor demand for the mill offals.

The proximate composition of rye flour is very similar to that of wheat flour; as in the instance of wheat, the principal proteins of rye are a prolamin and a glutelin with smaller quantities of an albumin and a globulin.[80, 81] The prolamin of rye (gliadin) appears to be identical in general properties and amino acid composition with that of wheat[82, 83] whereas the glutelin is not similar in its properties to wheat glutenin. It is impossible to wash gluten from doughs made entirely of rye flour and hence it is markedly inferior to wheat flour in baking properties; in the United States, most of the so-called rye bread is baked from mixtures of rye and wheat flour (usually a "first clear"). Rye flour contains a considerably higher percentage of a pectinlike carbohydrate gum than wheat flour and this is said to be partly responsible for the well-known stickiness of rye doughs. This carbohydrate gum appears to interfere with the formation of the gluten complex in mixtures of rye and wheat flours. If 35% rye flour is added to wheat flour, slightly more gluten can be obtained than from an equivalent quantity of the wheat flour alone; with increasing amounts of rye flour, the yield of gluten diminishes and when the mixture contains about 50% rye flour, no gluten can be obtained. The inferior baking properties of rye flour are in part attributed to this gum; acids such as lactic and acetic have a dispersing effect upon it which may explain the superiority of "sour-dough" rye bread over the yeast-leavened product.

Rye flour contains several times as much of a nonreducing trisaccharide sugar, trifructosan, as wheat flour and this difference has been employed as a chemical basis for determining the amount of rye flour admixtures with wheat flour.[84, 85]

3. Amylase Activity of Rye Flour in Relation to Baking Quality

The physical and chemical properties of rye flour and factors affecting its baking quality are dealt with in a treatise by Schulerud.[86] The control of α-amylase is of

---

[80] T. B. Osborne, *J. Am. Chem. Soc.*, **17**, 429 (1895).

[81] F. A. Csonka, and D. B. Jones, *J. Biol. Chem*, **82**, 17 (1929)

[82] T. B. Osborne, and S. H. Clapp, *Am. J. Physiol.*, **20**, 494 (1908)

[83] D B. Jones, C E F. Gersdorff, and O Moeller, *J. Biol. Chem.*, **62**, 183 (1924).

[84] C. I. Kruisheer, *Rec. trav. chim.*, **50**, 153 (1931).

[85] *Cereal Laboratory Methods.* 4th ed., Am. Assoc. Cereal Chemists, Lincoln, Neb., 1941.

[86] A. Schulerud, *Das Roggenmehl.* Moritz Schafer, Leipzig, 1939.

particular importance in the manufacture of breads made largely from rye flours.[86–88] Where there is insufficient α-amylase, the bread is characterized by a dry brittle crumb and the crust becomes cracked and torn upon cooling, whereas an excess results in bread with a wet soggy crumb which frequently pulls away from the crust leaving large hollow spaces in the bread. As α-amylase has an optimum activity at about 65° C., considerable dextrin formation may take place during oven baking before heat inactivation of the enzyme occurs. As the baking proceeds, the starch swells and undergoes partial gelatinization with a consequent increase in its hydration capacity, whereas the coagulation of the proteins results in a decrease in their water-holding capacity. The extent to which the starch is acted upon by α-amylase, therefore, has an important bearing on the characteristics of the bread crumb. In the quality control of rye flours in Europe, it has been necessary to develop technological tests for measuring α-amylase activity in relation to the changes it brings about during oven baking. One of these methods is based on a measurement of the viscosity of a flour–water suspension as the temperature is gradually raised. The increase in viscosity which takes place upon gelatinization of the starch is opposed by the liquefying action of the amylase and the maximum viscosity attained is an index of the amylase activity. Brabender[89] has described a recording torsion viscosimeter known as an amylograph which provides a continuous automatic record of the changes in viscosity of a flour–water suspension as the temperature is increased at a uniform rate. Since, in America, rye flours are almost invariably baked in mixtures with wheat flours, the amylase activity of the rye component is probably not of as great importance in relation to bread quality as in Europe. Moreover, the variations in amylase activity are doubtless considerably less than in northern Europe and in Scandinavia where there is considerable sprout damage as a result of wet harvest weather.

4. Definitions and Standards

Standards for rye flour have not been promulgated under the new Federal Food, Drug, and Cosmetic Act of 1938.

The following definitions have been approved by the Association of American Feed Control officials for rye by-products.

Rye Bran is the coarse outer covering of the rye kernel as separated from the cleaned and scoured rye.

Rye Feed, a by-product obtained in the usual process of the milling of rye flour from cleaned and scoured rye grain, consisting principally of the mill run of the outer covering of the rye grain and the germs, with small quantities of flour and aleurone.

Rye Flour Middlings consists of the rye feed, rye red dog, and pure dark rye flour combined in the proportions obtained in the milling of rye flour, and must not contain more than 5% of crude fiber.

Rye Low-Grade Feed Flour consists principally of dark rye flour and small quantities of aleurone and fine bran particles, and must not contain more than 1.5% crude fiber.

---

[87] G. Molin, *Cereal Chem.*, **11**, 153 (1934).

[88] C. W. Brabender, G. Mueller, and A. Köster, *Z. ges. Getreide-, Mühlen- u. Bäckereiw.*, **24**, 168 (1937).

[89] C. W. Brabender, *Mühlenlab.*, **7**, 121 (1937).

Rye Middlings consists of the rye feed and the rye red dog combined in the proportions obtained in the usual process of milling rye flour.

Rye Red Dog, a by-product obtained in the usual process of the milling of rye flour, consists principally of aleurone with small quantities of flour and fine bran particles, and must not contain more than 3.5% crude fiber.

IX. WHEAT

1. Utilization

The principal use of wheat in the United States is for food; approximately two-thirds of the annual domestic consumption, or about 480,000,000 bu., are processed for this purpose. Until 1941, very little wheat (probably about 1,000,000 bu.) was used annually for industrial purposes. The limited use of wheat as a food and for industrial purposes is due mainly to its high price in relation to other grains.

The main use of wheat for food purposes is in the manufacture of flour for making bread, biscuits, and pastry products; a small portion is converted into breakfast cereals and into raw materials for the macaroni-making industry. Large quantities of flour are not sold directly in the form in which they come from the mill, but are utilized in the production of blended and prepared flours. The production and value of various types of flour, breakfast foods, and mill offals made from wheat in 1939 are shown in Table 135.

TABLE 135

U. S. PRODUCTION AND VALUE OF FLOUR, BREAKFAST FOODS, AND MILL BY-PRODUCTS MADE FROM WHEAT IN 1939

Products	1000 Pounds	Value
White flour, for sale as such	21,828,270	$458,356,323
White flour, blended, plain	142,499	3,220,955
White flour, phosphated	726,975	15,942,608
White flour, self-rising	1,328,261	32,080,551
White flour, ready-mixed	386,693	24,352,238
Graham and whole wheat flour	428,662	8,880,384
Semolina	665,184	13,423,319
Total flour and semolina	25,506,544	$556,256,378
Breakfast foods:		
Ready to serve	299,539	$37,665,239
To be cooked before serving	153,453	10,463,153
Total	452,992	$48,128,392
Bran and middlings	8,999,846	$91,896,004

The production of wheat flour is by far the largest milling industry in the United States. Data compiled by the Department of Commerce from reports by mills which normally produce 5000 bbl. or more of flour annually show that for the 10-year period, 1930–1939, these mills ground an annual average of 471,800,000 bu. of wheat with an output of 102,360,000 bbl. of flour and 8,355,139,000 lbs. of mill feed. The State of Kansas leads in flour output followed by New York and Minnesota; these three States account for about 38% of the entire output. The leading milling center is Buffalo, with Kansas City second, and Minneapolis third. Other important milling centers in order of decreasing output are Salina, Toledo, Tacoma, Wichita, Portland, Seattle, Chicago, and St. Louis. According to the *Northwestern Miller* (Minneapolis) there were 3001 active or potentially active flour mills in 1942 with a total capacity of 709,768 bbl. of flour per 24 hrs. The largest single milling organization has a daily capacity of 70,650 bbl. of flour.

The quantity of durum wheat milled annually has varied from about 11.5 to 17.5 million bu. from 1935 to 1941. In 1941, 17,523,678 bu. were milled with a production of 2,857,844 bbl. semolina and 929,559 bbl. flour (one barrel, 196 lbs). The majority of the durum mills are located in Minneapolis and St. Paul.

The per capita consumption of flour has declined from 224 lbs. in 1900 to 154 lbs. in 1933–1934 and has remained stable at the latter figure during recent years; this marked decrease in consumption over a period of years has been due largely to the increased use of other foods and to the reduced energy requirements as a result of the more extensive use of machines.

The industrial uses of wheat include the manufacture of malt, potable spirits, industrial alcohol, starch, gluten, pastes, and core binder. Because of its relatively high price, wheat malt has not been employed to any extent in the brewing and distilling industries; its chief use is by the flour-milling industry for increasing the amylase activity of high-grade flours. Until 1942, the quantity of wheat used in the production of industrial alcohol and potable spirits rarely exceeded 51,000 bu. per annum; considerable quantities of surplus wheat held by the Government are now being sold at relatively low prices for conversion into ethyl alcohol in order to meet the increased demands resulting from the current war. Small quantities of wheat flour (chiefly low-grade flours) are used in the manufacture of wheat starch and gluten. Low-grade flours are used to some extent in the manufacture of pastes for use in book binding and in paper hanging, in the manufacture of plywood adhesives and in iron foundries as a core binder in the preparation of molds for castings.

For many years, the production of wheat has exceeded domestic utilization and since 1932 the export demand has been very limited with the result that large surpluses have accumulated. Under the Agricultural Adjustment Act of 1938, the Federal Government has established four Regional Research Laboratories with the object of developing new uses for farm commodities. It may be anticipated that wheat will find increasing industrial uses in the future; the extent of this development will depend in large measure on the relative cost of wheat as an industrial raw material; this in turn depends chiefly on the demand of wheat for use as a food.

2. Common Wheat and Its Mill Products

(a) *Wheat and Flour Quality*

In wheat and flour technology, the term quality is a purely relative one and denotes the suitability of the material for some particular purpose; it has no reference to nutritive values. Thus, amber durum wheats possess characteristics which render them particularly suitable for the production of raw material for macaroni manufacture, but durum wheat flour is of low quality for bread and cake manufacture. Similarly, hard wheat flour is of high breadmaking quality but is inferior to soft wheat flours for chemically leavened products such as biscuits, cakes, and pastry. Technologically, the quality of the common or *vulgare* wheats (and also of the club or *compactum* wheats) involves many factors which are conveniently classified into two groups: Those associated with milling quality; and those related to the value of the flour for specific uses.

Wheat-milling quality is concerned with the milling characteristics of the wheat and the yield of refined flour which can be obtained. The miller naturally desires a wheat which will mill easily and give a high flour yield. The wheat kernels should be plump and uniformly large in size to permit ready separation of foreign material without loss of millable wheat. The wheat should absorb water readily and uniformly in the tempering process and produce a high yield of flour with a maximum and clean separation from the bran and germ without undue consumption of power.

As the density of the endosperm is greater than that of the bran and the germ, the greater the weight of a bushel of clean wheat at a given moisture content, the higher, in general, is the flour yield. Certain varieties and also wheats which have been weathered by exposure to intermittent rains during harvest give flour yields which are out of line with their test weights; weathering lowers test weight per bushel because of swelling of the kernel although the proportion of endosperm remains the same. Varietal and environmental factors also influence the ease of milling; the bran of weathered and frosted wheats tends to pulverize and it is difficult to secure a clean separation of the flour from the bran.

While there are many complex factors involved in flour quality, the quantity of proteins and their physical properties are of prime importance. The endosperm proteins of wheat, gliadin and glutenin, possess the peculiar and distinctive property of forming gluten when wetted with water. The presence of gluten in wheat-flour dough imparts physical properties which differ from those of doughs made from any of the other cereal grains; in fact, it is to this capacity of gluten formation rather than to any distinctive nutritive properties, that wheat owes its prominence in the diet of the white races. When water is added to wheat flour, the proteins hydrate and form gluten, a complex coherent mass, in which starch, yeast, and other dough components are embedded. Thus, the gluten is, in reality, the skeleton or framework of wheat-flour dough and imparts the property of gas retention which makes possible the production of light leavened products. Crude wet gluten is readily obtained by kneading wheat-flour dough in a stream of water to wash out the starch and other removable components. It normally consists of about two-thirds water but the balance by no means consists entirely of protein. Certain of the flour components, including a portion of the lipids, enzymes, and electrolytes, are adsorbed on the gluten and cannot be removed even by prolonged washing; some starch also remains as an impurity so that dry gluten normally contains about 70 to 80% protein. The percentage of dry gluten bears a close relation to the percentage of protein and before the Kjeldahl method for determining nitrogen came into general use, gluten determinations were widely employed as an index of the value of the flour for baking purposes.

Aside from variations in the quantity of gluten obtainable from different flours, marked differences are found in its physical properties. Hard wheat flours, such as those milled from red spring and hard red winter, yield relatively large quantities of a coherent tough, ductile, tenacious gluten which is elastic and can be stretched out considerably without tearing or breaking; on the other hand, soft wheat flours, such as soft red winter, white club, and many of the white wheat flours yield relatively small quantities of gluten which is soft, lacks coherence, and cannot be stretched appreciably without tearing. The proteins of soft flours hydrate, or form gluten, more rapidly than those of hard flours. In common with other hydrophilic colloids, the physical properties of gluten are influenced by many factors, such as mechanical manipulation, time, temperature, salt concentration, and *p*H, which come into play in the manufacture of leavened baked products. Thus in bread manufacture, the physical properties of the dough are influenced by the extent of mixing it receives, and, during yeast fermentation, the acidity which develops, "mellows" or "ripens" the gluten. Certain oxidizing agents, such as ammonium persulfate, nitrogen trichloride potassium bromate, and potassium iodate, even in very low concentrations, have a marked influence on gluten properties. While the mechanism of their action is not well understood, they have the effect of decreasing the mechanical treatment and the fermentation required to bring the gluten into the optimum condition for baking.[90] Such substances are frequently employed as "flour improvers," and in proper concentrations under appropriate baking conditions their use frequently results in bread of greater volume than can be obtained in any other known manner; this is particularly true in the instance of high-protein flours.

The value of a wheat flour for a particular baking purpose depends primarily on the quantity of protein present and the colloidal characteristics of the gluten which is formed upon making a dough. On the basis of their suitability for the manufacture of yeast-leavened bread, common, or *vulgare*, wheats and the flours milled therefrom are classified broadly into two groups: hard or strong; and soft or weak. Hard or strong wheat flours contain a relatively high percentage of protein which forms a tenacious, elastic gluten of good gas-retaining properties and are capable of being baked into well-risen shapely loaves possessing good crumb grain and texture. They require a relatively large amount of water to make a dough of the proper consistency and hence give a high yield of bread. The doughs have excellent "handling qualities" and are not critical in their mixing and fermentation requirements; for this reason, they will yield good bread over a relatively wide range of baking conditions and are said to have good fermentation tolerance. In contrast, "soft" or "weak" flours have a relatively low protein content and form a soft, weak, relatively nonelastic gluten of poor gas-retaining properties. They have relatively low water-absorbing capacity, yield doughs of inferior "handling quality" which give trouble in machine baking; and are very critical in their mixing and fermentation requirements so that they are more likely to give baking failures. Weak flours require less mixing and fermentation than strong flours to give optimum baking results.

---

[90] Ting Shen and W. F. Geddes, *Cereal Chem.*, 19, 609 (1942).

American bakery practice and the quality demands of the consumer in the United States dictate the use of strong relatively high protein flours for breadmaking purposes. Of the commercial market classes listed in Table 113, page 603, the hard red spring and hard red winter wheats are eminently suitable for the production of yeast-leavened bread. In general, the hard red spring wheats are the stronger but the division between these two classes is not sharp, and many lots of hard winter wheat are superior to the average spring wheat. White wheats vary widely in baking quality, depending on the variety and where they are grown. From certain areas they yield satisfactory bread flours; this is particularly true of Early Baart, Pacific Blue Stem, and Hard Federation grown in portions of eastcentral Washington. While the hard wheats are of higher quality than the soft wheats for breadmaking purposes, the reverse is true for the manufacture of chemically leavened products. In such bakery goods, there is no fermentation to "mellow" or "ripen" the gluten, and the low protein content and the soft, mellow characteristics of the gluten formed by soft or weak flours produce a lighter, more tender product than strong flours.

Within the strong and weak classes varying degrees of strength or weakness are required by the bakery trade. Thus, the general strength of bread flours sold for the family trade, for commercial "pan" bread, and for commercial "hearth" bread must increase in the order named. In home baking, the whole procedure involves rather mild treatment (hand or very slow-speed mixing and gentle fermentation), so that good results are obtained with a flour of lower protein content and more easily conditioned gluten than would be satisfactory for commercial bakeries. For the manufacture of pan bread, a medium strong flour is required to withstand high-speed mixing and produce a dough possessing the physical characteristics which will permit machine manipulation without difficulty. For bread baked on the hearth of the oven without pans, a flour of still higher protein content which yields a strong dough is desired in order that the dough will not flatten unduly under its own weight. Other types of bread flours must be supplied by the flour miller to suit different markets.

In the instance of soft wheat flours, the protein content and gluten "quality" desired also vary over a relatively wide range. For example, in soda cracker manufacture, different types of flours (depending upon whether they are used in the sponge or as doughing flours) varying in protein content from about 9 to 10% are required. For cake making, very weak flours, with protein contents ranging from about 7 to 9% and which yield doughs having a *p*H of 5.1 to 5.3 give the best results.

This very general treatment of flour strength may lead the reader to surmise that the utility of a flour for any purpose may be readily and accurately determined by making a quantitative protein test and measuring the colloidal characteristics of the gluten or the doughs. Indeed, much research has been devoted to determining gluten and dough characteristics.[91, 92] Baking quality has been found to depend upon so many factors

---

[91] D. W. Kent-Jones, *Modern Cereal Chemistry*. 3rd ed., Northern Pub. Co., Liverpool, 1939.

[92] C. H. Bailey, *Wheat Studies, Food Research Inst.*, **16**, 243 (1940).

which vary from flour to flour, that no single test has yet been found to be a completely reliable index of the behavior of a flour under any particular set of baking conditions. For this reason it has been necessary to devise standard experimental baking tests for bread, biscuits, cakes, pies, etc.[93] The results of such tests must be interpreted in terms of the conditions under which the flour is to be used commercially, and this requires no little skill and experience.

Quality factors other than those related to protein content and gluten "quality" must be considered by the flour miller; for example, in the instance of bread flours, he must insure that there is adequate amylase activity to produce sufficient sugar from the starch to support yeast (fermentation) throughout the entire fermentation. Otherwise, under some baking conditions, failures will result from insufficient "aeration" of the dough. On the other hand, too high an amylase activity, as in the instance of flour milled from sprouted wheat, is undesirable since the excessive production of dextrins from the starch causes the dough to slacken during fermentation[94] and to yield bread with a tough, soggy crumb.[95] The flour miller maintains the amylase activity at the desired level by wheat selection and the use of small quantities of malted wheat flour where necessary.

Another factor which the miller must control is the yellow pigment content of the flour, since the majority of the American bread-eating public demands bread with a relatively white crumb. This consumer requirement is met by the use of very small quantities of bleaching agents which convert the carotenoid pigments of the flour to colorless compounds. The enrichment of flour with certain vitamins and minerals, discussed on page 662, has introduced an additional set of variables which the miller must control in the flour he manufactures.

From what has been said, it is clear that scientific and technological advances have required the miller to exercise an increasing measure of scientific control in order to produce flour which will meet the rigid and highly specialized requirements of the baking trades.

(b) *Chemical Composition of Mill Streams and Products*

The data secured by B. R. Jacobs in a study of the streams from a large Minneapolis mill and published by Bailey[96] are reproduced in part in Table 136 to illustrate the general trends in proximate composition. In progressing from the head to the tail end of the mill (see Vol. II, Chapter XV), the flour streams contain progressively more of the components which are characteristic of the bran and germ. Various analyses have shown that the ash content of the bran is from 20 to 25 times, and that of the germ from 10 to 15 times the ash content of the endosperm. The ash content of a flour therefore gives a reasonably accurate criterion of the relative freedom from bran and germ, although some variation naturally occurs in flours of equivalent purity milled from different wheats.[97] The protein content of bran is only about 1.4 times that of the endosperm as a whole, so that, as the "purity" of the flour decreases, the ash content rises much more sharply than the protein content. The presence of the increasing amounts of bran and germ decreases the quality of the flour for baking purposes and a

---

[93] *Cereal Laboratory Methods.* 4th ed., Am. Assoc. Cereal Chemists, Lincoln, Neb. (1941).

[94] F. C. Hildebrand and G. M. Burkert, *Cereal Chem.*, **19**, 27 (1942).

[95] D. W. Kent-Jones and A. J. Amos, *Cereal Chem.*, **17**, 265 (1940).

[96] C. H. Bailey, *The Chemistry of Wheat Flour.* Chemical Catalog Co., New York, 1925.

TABLE 136

CHEMICAL COMPOSITION OF CERTAIN MILL STREAMS AND BY-PRODUCTS OBTAINED IN WHEAT MILLING[a]

Product	Moisture, %	Total nitrogen, %	Fat, %	Fiber, %	Pentosans, %	Ash, %	Total sugars, %	P_2O_5, %
Wheat and mill products:								
Wheat	10.3	2.05	2.1	..	5.1	1.73	2.6	0.85
First patent flour	11.5	1.82	1.0	0.2	2.9	0.40	1.3	0.22
First clear flour	11.0	2.13	1.7	0.2	3.6	0.81	1.8	0.44
Second clear flour	10.4	2.33	2.0	0.3	3.4	1.34	2.1	0.70
Red dog	9.2	2.87	5.4	2.4	8.4	3.15	6.4	1.63
Bran	8.8	2.33	4.1	10.8	25.1	6.38	5.4	3.15
Shorts	8.9	2.47	5.2	8.4	16.3	4.10	6.0	2.23
Germ	8.5	4.84	11.9	1.8	6.2	4.80	15.1	2.73
Flour streams:								
First break flour	11.8	1.91	1.1	0.2	3.3	0.66	1.4	0.34
Second break flour	11.3	1.99	1.4	0.1	3.3	0.56	1.3	0.28
Third break flour	11.5	2.08	1.4	0.1	2.6	0.49	1.4	0.26
Fourth break flour	11.2	2.29	2.2	0.1	2.9	0.64	1.5	0.35
Fifth break flour	11.0	2.35	2.6	0.1	2.8	1.03	1.6	0.55
First middlings flour	11.5	1.80	1.0	0.1	2.6	0.36	1.2	0.19
Third middlings flour	11.1	1.80	1.1	0.1	3.0	0.38	1.4	0.20
Fifth middlings flour	10.7	1.89	0.9	0.1	3.0	0.44	1.5	0.24
Seventh middlings flour	11.1	1.96	1.4	0.1	3.4	0.65	2.5	0.36
Ninth middlings flour	10.8	1.84	1.5	0.2	3.0	0.61	2.0	0.32
First tailings from purifier	9.8	2.57	5.4	4.4	11.5	3.67	4.3	1.85

[a] Condensed from C. H. Bailey, *Chemistry of Wheat and Wheat Products*. Chemical Catalog Co., New York, 1925.

TABLE 137

AVERAGE PROTEIN AND ASH CONTENTS OF VARIOUS GRADES OF HARD WHEAT FLOURS[a]

Flour grade	Proportion of total flour, %	Hard red spring Minnesota		Hard red winter Kansas and Nebraska		Semihard white Blue Stem	
		Protein, %	Ash, %	Protein, %	Ash, %	Protein, %	Ash, %
Family patent[b]	70–75	11.0	0.39	11.0	0.38	..	..
Short patent	75–80	12.6	0.40	11.9	0.40	10.8	0.38
Medium patent	80–85	12.8	0.43	12.1	0.42	11.0	0.40
Long (standard) patent	90–95	13.0	0.45	12.3	0.44	11.2	0.44
Straight grade	...	13.5	0.47	12.5	0.46	11.4	0.46
First clear, fancy	...	..	..	13.9	0.65	..	..
First clear	...	14.8	0.70	..	..	..	..

[a] Courtesy of W. E. Long Co., Chicago.

[b] Family patent is made from a wheat mix of lower protein content than the other grades of hard wheat flour.

---

[97] R. C. Sherwood and C. H. Bailey, *Cereal Chem.*, 5, 437 (1928).

maximum limit of one-twentieth of the protein plus about 0.3% (0.35% when the calculations are made on a moisture-free basis) has been established by the Federal Government as the value above which flours are unsuited for the production of white bread and biscuits.

TABLE 138

DISTRIBUTION OF B VITAMINS, CALCIUM, AND IRON IN PRODUCTS OF WHEAT MILLING

Product	Thiamine[a]			Riboflavin[b]		Niacin[c]	
	Mill yield, %	γ per g.	Per cent of total in mill fraction	γ per g.	Per cent of total in mill fraction	γ per g.	Per cent of total in mill fraction
Patent flour	63.0	0.68	8.0	0.34	20.5	12	10.2
First clear flour	7.0	3.00	3.9	0.62	3.2	26	3.2
Second clear flour	4.5	12.37	10.0	1.85	7.7	83	4.7
Red dog flour	4.0	29.66	22.0	3.80	14.1	120	7.6
Germ	0.2	22.93	0.9	..	...	68	0.2
Shorts	12.3	17.40	39.6	2.80	32.5	159	17.8
Bran	9.0	9.37	15.6	2.80	22.0	330	56.3
Wheat	100.0	5.03	100.0	1.00	100.0	70	100.0

Product	Niacin[d]		Pantothenic acid[d]		Pyridoxine[d]	
	γ per g.	Loss in milling, %	γ per g.	Loss in milling, %	γ per g.	Loss in milling, %
Patent flour	10	83	5.7	57	2.2	52
First clear flour	21	64	9.6	28	3.9	15
Second clear flour	57	3	12.8	4	5.7	..
Wheat germ	34	..	15.3	..	9.6	..
Wheat	59	..	13.3	..	4.6	..

Product	Calcium[e]			Product	Iron[g] (dry matter basis)	
	Mill yield, %	Calcium (as Ca), %	Calcium in ash (as CaO), %		Iron as (Fe), γ per g.	Iron in ash, %
Patent flour	58.0	0.018	5.2	Patent flour	8.4	0.18
Clear flour	12.0	0.023	3.9	First clear flour	17.4	0.22
Low-grade flour	2.6	0.038	3.6	Second clear flour	38.7	0.26
				Red dog	96.2	0.30
Middlings, total mill run	14.4	0.112	3.4	Shorts	139.0	0.28
Bran	13.0	0.116	2.4	Bran	146.2	0.21
Germ	[f]	0.069	1.9	Germ	91.3	0.19
Wheat	100.0	0.045	2.8	Wheat	41.6	0.23

[a] Average data of R. C. Sherwood, R. Nordgren, and J. S. Andrews, *Cereal Chem.*, **18**, 811 (1941).

[b] Average data of J. S. Andrews, H. M. Boyd, and D. E. Terry, *Cereal Chem.*, **19**, 55 (1942).

[c] Average data of J. S. Andrews, H. M. Boyd, and W. A. Gortner, *Ind. Eng. Chem., Anal. Ed.*, **14**, 633 (1942).

[d] Average data of L. J. Tepley, F. M. Strong, and C. A. Elvehjem, *J. Nutrition*, **24**, 167 (1942).

[e] B. Sullivan and C. Near, *Ind. Eng. Chem.*, **19**, 498 (1927).

[f] The germ was diverted to the middlings; the analyses given are for a separate sample of germ taken from the same mill run.

[g] J. S. Andrews and C. Felt, *Cereal Chem.*, **18**, 819 (1941).

The chemical composition of different grades of flour milled from various classes of wheat is given in Tables 126 and 137.

Data on the distribution of B vitamins, calcium, and iron in wheat mill products are assembled in Table 138; the relation between the per cent extraction and the concentration of thiamine, riboflavin, nicotinic acid (niacin), and pantothenic acid as given by Andrews,[98] is represented graphically in Fig. 57. These data indicate that patent flours of 58 to 65% extraction contain about 8% of the thiamine, 20% of the riboflavin, 10% of the nicotinic acid, 23% of the calcium, and 12% of the iron present in the wheats from which they were milled. When the extraction is increased above about 75% by adding successively less and less highly purified mill products, there is a sharp increase in the levels of these B vitamins. A higher proportion of the total pantothenic acid and pyridoxine of the wheat is found in the patent flour than in the instance of the other vitamins mentioned. The distributions of the vitamins and minerals in the wheat kernel structures, therefore, do not parallel each other. While thiamine and riboflavin are somewhat similarly distributed, there is a larger percentage of the latter in the endosperm. Bran is the best source of nicotinic acid and iron whereas shorts are the highest of all products in thiamine. It is of importance to note that, in order to raise the thiamine content of patent flour to the whole wheat level, it would be necessary to add 12 to 15% of wheat germ, a quantity which would ruin the baking quality.

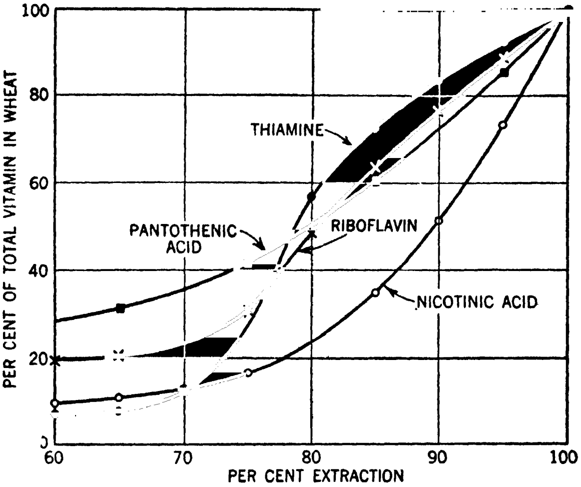


Fig. 57.—Distribution of B-vitamins in products of wheat milling.[98]

As a result of variations in the distribution of the B vitamins in the wheat kernel, lengthening of the extraction will not produce the same increase in the levels of the various B vitamins present in the flour.

The distribution of vitamin E in hard wheat and its mill products as found by Binnington and Andrews[99] is shown in Table 139. From their data, these workers compute that about 55% of the vitamin E of this class of wheat is located in the germ and the remainder in the endosperm layers closely adjacent to the bran.

---

[98] J. S. Andrews, *Food Industries*, **15**, No. 878 (1943).

[99] D. S. Binnington and J. S. Andrews, *Cereal Chem.*, **18**, 678 (1941).

In view of the variations in the proximate composition, vitamin, and mineral contents of different wheats, the levels of these constituents in different flours of equivalent purity are subject to variation. The proximate chemical composition of different wheat products used for food purposes is shown in Table 126 and the average levels of B vitamin and selected minerals in Table 127; the average composition of mill products used for animal feeding is recorded in Table 129.

TABLE 139

DISTRIBUTION OF TOCOPHEROL IN HARD-WHEAT MILL PRODUCTS[a]

Product	Mill yield, %	Oil content, %	Tocopherol per 100 g.		Percentage of total tocopherol in mill fraction, %
			In oil, %	In mill product, mg.	
Patent flour	60.3	0.83	0.003	0.03	2.0
First clear flour	9.4	1.78	0.082	1.46	17.4
Second clear flour	4.1	4.16	0.069	2.87	14.8
Red dog flour	2.7	5.83	0.099	5.77	20.0
Shorts	9.3	4.41	0.072	3.18	37.6
Bran	14.0	2.97	0.012	0.30	6.3
Germ	0.1	8.90	0.178	15.84	1.9
Whole wheat	..	1.54	0.059	0.91	..

[a] D. S. Binnington and J. S. Andrews, *Cereal Chem.*, 18, 678 (1941). The patent flour was bleached and it has subsequently been learned that this causes considerable destruction of tocopherol (private communication).

(*c*) *Bromated White Flours*

For certain markets, strong high-protein flours containing potassium bromate are best suited for the type of bread desired and the particular baking methods employed. Thus, in Spanish-American countries, the prevailing type of bread is hearth baked and a strong flour is required to produce a dough which will retain a more or less cylindrical shape during baking. As relatively small quantities of yeast are employed in a rather long fermentation, the addition of potassium bromate aids materially in the proper "ripening" of the gluten and thereby in the production of optimum bread. Flours for this trade usually contain around 14% protein (13.5% moisture basis) and about 20 to 50 p. p. m. of potassium bromate depending upon the crop characteristics and the particular trade.

Within the United States, relatively rich, Vienna-type rolls (so-called Kaiser rolls) are particularly popular with the Jewish trade in the East. Flours for this market normally contain from 14.5 to 15.5% protein (13.5% moisture basis) and from 3 to 10 p. p. m. of potassium bromate; as the dough contains 2 to 3% yeast and is given several hours' fermentation, very much less potassium bromate is required than in the instance of flours for the Spanish-American market.

Bromated white flours are not usually sold to the retail trade in the United States.

(*d*) *Whole Wheat or Graham Flours*

Whole wheat flours (entire wheat flour or graham flour) are commonly used for making whole wheat bread; for this purpose they must be made from hard or bread wheats which are ground to a fairly fine flour. Normally, whole wheat flours are of such fineness that at least 90% will pass a No. 8 sieve and 50% will pass a No. 20 sieve. Whole wheat flours are frequently made by recombining the mill streams obtained in the gradual reduction roller milling process (see Vol. II, Chapter XV) in the proper proportions. The flour streams may be combined and bleached in the usual manner before adding the other finely ground streams. Malted wheat flour or malted barley flour may be added where necessary to raise the amylase activity to a satisfactory level. Potassium bromate may also be added to whole wheat flours at levels not exceeding 75 p. p. m. to improve their baking properties.

(*e*) *Blended and Prepared Flours*

Large quantities of flour are not sold directly to the domestic trade in the form in which they come from the mill, but are utilized in the production of several forms of blended and prepared flours. The blended flours comprise plain-blended, phosphated, and self-rising flour, while the prepared flours include ready-prepared mixes for the production of biscuits, cakes, doughnuts, and pancakes. These products are made as secondary products by the flour-milling industry and also by special establishments which purchase all the ingredients. The magnitude of this industry in 1939 is shown in Table 135 compiled from the last U. S. Census Report covering these products.

Plain-Blended Flours.—Plain-blended flours comprise mixtures of flours made from various classes of wheat, such as hard red winter wheat and hard white wheat, and represents a relatively minor fraction of the total production of blended flours.

Phosphated Flours.—Phosphated flours represent a very large percentage of the so-called family flours which are marketed in the southeastern States and comprise bleached soft wheat flours to which monocalcium phosphate has been added. These flours are designed for use with soda and sour milk in the making of sour milk biscuits. When made with plain flour, such biscuits frequently have a yellow color and soda taste because of insufficient acidity in the milk to react with all the soda; the addition of monocalcium phosphate provides an acid-reacting material to neutralize the excess soda. The proportions recommended in the trade are 0.5 lb. monocalcium phosphate monohydrate, $CaH_4(PO_4)_2 \cdot H_2O$ (or 0.375 lb. of the anhydrous salt), to each 100 lbs. of flour.[100] The commercial production of the anhydrous salt is a recent development. It dissolves slowly in water so that there is little reaction with soda during the initial stages of mixing biscuit doughs; this delay in maximum rate of carbon dioxide production results in a very efficient leavening action.

The phosphate is incorporated with the flour either by means of a batch mixer or by a continuous method in which the chemical is generally fed into the flour stream at the bleaching agitator, by means of a special feeder. Where the flour is to be bleached with a powdered bleaching agent, such as Novadelox, many mills add both ingredients from the same feeder, by making up a "master mix" of the chemicals with flour.

---

[100] R. A. Barackman, Victor Chemical Co., Chicago Heights, Ill., private communication.

Self-Rising Flours.—Self-rising flours are in large demand in the South. They are made from bleached, soft wheat patent flours and contain salt, sodium bicarbonate, and an acid-reacting ingredient (monocalcium phosphate) in the proper proportions for the production of chemically leavened biscuits. Effective leavening requires a minimum carbon dioxide production of 0.5% of the weight of the flour. In order to avoid a yellow color and a disagreeable "soda taste" (somewhat characteristic of soap), sufficient acid ingredient must be added to react with all the soda.

Even relatively pure salt is somewhat hygroscopic and under conditions of high atmospheric humidity the salt crystals tend to collect a film of flour around them and form "shot balls" or "dough balls." The use of fine salt containing an appropriate drier (such as tricalcium phosphate), coupled with thorough mixing, greatly reduces trouble from this condition. The sodium bicarbonate must also be in finely divided form, otherwise "soda specks" or brown spots will be formed on the crust of the baked biscuits. Soda manufacturers furnish a grade of free-flowing sodium bicarbonate containing about 0.5% tricalcium phosphate, which is so fine that about 75% of it will pass through a 200-mesh screen.

The calcium acid phosphate must be of high purity, uniform "neutralizing strength," and correct granulation, to facilitate mixing with the flour and to give the proper rate of reaction with the soda. The neutralizing strength of various brands differs somewhat but averages around 80% for the monohydrate; the anhydrous salt has a neutralizing strength of about 83.3%; that is, 100 lbs. of these salts will neutralize 80 and 83.3 lbs. of sodium bicarbonate, respectively.

A common formula for the production of self-rising flours contains 1.5 parts sodium bicarbonate, 1.875 parts monocalcium phosphate monohydrate, and 1.75 parts by weight of salt to each 100 parts by weight of flour.[100] Where the anhydrous phosphate is used, a formula recommended by the manufacturers embodies the addition of 1.25 parts sodium bicarbonate, 1.5 parts anhydrous monocalcium phosphate, and 2 parts salt to each 100 parts flour. Because of the slower rate of solution of the anhydrous phosphate, less carbon dioxide is lost during the formation of the dough, so that slightly less soda is required to obtain adequate leavening than when the monohydrate is employed.

In mixing self-rising flours, it is, of course, essential that the soda and phosphate do not come in direct contact. The salt and phosphate are first added to the flour and the batch mixer operated for 4 to 5 min.; the soda is then introduced and the mixing continued for a further 15 to 20 min.

Enriched Self-Rising Flours.—Enriched self-rising flours are prepared to conform with the federal regulations outlined on page 668. In order to insure optimum retention of thiamine in the baked biscuit, Barackman[101] has shown that it is desirable to increase slightly the quantity of monocalcium phosphate above that employed in the standard commercial self-rising flour formulas. The standard self-rising flour formulas give *p*H values in baked biscuits of from 7.1 to 7.3, depending chiefly upon whether they are made with water or milk. About 85% of the added thiamine survives if the *p*H of the baked product is 7.1 or lower. In present commercial practice, the standard quantities of the monohydrate and anhydrous phosphate are commonly increased by 0.125% when making the enriched product.

---

[101] R. A. Barackman, *Cereal Chem.*, 19, 121 (1942).

Ready-Mixed Flours.—Ready-mixed flours for the production of biscuits, cakes, doughnuts, pancakes, and the like are on the market. These preparations contain all the necessary ingredients other than the liquid (water or milk) in the proportions required to make the product. The various ingredients in such mixes perform definite functions and it is essential that the formula be properly "balanced" in order to produce baked products of optimum quality. Thus, individual ingredients may be characterized as strengtheners or tougheners, tenderizers or enricheners, aerators, and flavorers. The chief function of flour is to act as a strengthener or structure builder and it therefore falls under the heading of a toughener; sugar is a flavorer, and enrichener, or tenderizer. Shortening is also an enrichener or tenderizer and contributes to the keeping quality of the baked product, but an excess "weakens" the dough structure. Dried egg yolks function as tenderizers because their high fat content more than offsets the toughening action of the protein. Dried whole egg or egg whites, on the other hand, are strengtheners or tougheners. Skim milk solids are tougheners; they affect the crust color and symmetry of the product and are an important "governor" in the formula. Leavening functions, of course, as an aerator.

In the manufacture of ready-prepared mixes containing shortening, the fat must be of a type that will not readily become rancid; this requirement is met by the use of vegetable shortenings prepared from vegetable oils of high stability. The incorporation of the shortening with the other finely divided "dry" ingredients presents a difficult technical problem but special mixers have been developed for this purpose; some manufacturers purchase a powdered preparation of shortening in dried milk.

In ready-mixed preparations the acid-reacting ingredient for leavening is frequently sodium acid pyrophosphate and for many products is said to give superior volume and texture to those obtained with monocalcium phosphate.[102] Since, however, the pyrophosphate leaves a distinctly alkaline residue, monocalcium phosphate is frequently used in conjunction with it in the preparation of mixes yielding white baked products. The addition of the monophosphate results in baked goods of lower *p*H and thereby decreases the tendency toward the development of a yellowish-white crumb. The development and maintenance of properly balanced formulas require considerable experimental work and technical control.

Prepared biscuit mixes are made by incorporating from 12 to 17 lbs. shortening, 1 to 3 lbs. dry skim milk, 1 to 3 lbs. sucrose, and 2 lbs. salt, together with the required amount of soda and phosphates, with each 100 lbs. soft wheat flour.

---

[1] J. R. Chittick, *Am. Soc. Bakery Engrs., 1940 Proceedings*, p. 247.

A typical prepared doughnut[108] flour contains 20 lbs. sucrose, 4 lbs. dried whole eggs, 5 lbs. dried skim milk, 5 lbs. hydrogenated shortener, 2 to 4 lbs. leavening agents (preferably a slow-acting combination), 0.5 lb. flavoring (mace or nutmeg, vanilla, lemon, etc.), and 1 lb. salt with each 100 lbs. of flour. In general, a relatively long extraction and coarsely granulated soft winter wheat flour containing not over 10% protein (15% moisture basis) is used as the base flour in making doughnut mixes. Blending of hard wheat flour with soft wheat flour is sometimes practiced, particularly if the formula is relatively high in sugar and shortening. As a means of obtaining a short, tender doughnut, 5% or more of the flour is frequently replaced with corn starch, potato starch, or cottonseed flour.

Self-rising pancake flours may be prepared by mixing 2 parts sodium bicarbonate, $2^1/_2$ parts monocalcium phosphate, 2 parts salt, and 100 parts by weight of a flour blend. The flour blend comprises various mixtures of wheat flour, corn flour, and rice flour but wheat flour usually makes up at least 50% of the mixture. Powdered buttermilk, powdered skim milk, or corn sugar is also frequently added in quantities of from 5 to 7%. In buckwheat pancake flours, the quantity of sodium bicarbonate is commonly increased by 0.2 part and the salt by 0.25 part over the amounts given above.

(*f*) *Coarsely Ground Wheat Mill Products*

Three types of granular wheat products, crushed, or coarse ground wheat, cracked wheat, and farina, are made from common wheat.

Crushed wheat or coarsely ground wheat is made by grinding or crushing cleaned wheat between coarsely corrugated rolls similar to the break rolls employed in milling flour. Cracked wheat is made on Le Page corrugated rolls; the fast roll is corrugated longitudinally and the slow roll transversely, so that the wheat is subjected to a cutting action. This results in the production of angular-shaped particles of more uniform, and, as made, of smaller size than those obtained by crushing wheat. These products are used as components of special breads and as breakfast cereals.

Farina consists of coarsely ground uniform-sized particles of highly purified fragments of the endosperm of *vulgare* wheats and is used as a breakfast cereal and in the manufacture of macaroni products. Enriched farina is prepared to conform with Federal Definitions and Standards for this product. In order to accelerate the gelatinization of the starch during cooking, disodium phosphate may be added.

(*g*) *Enrichment of Flour and Cereal Products*

Background.—Recent investigations of the diets of the American people (as well as those of other countries) have established that serious and widespread deficiencies exist in all age groups in regard to the intake of thiamine, riboflavin, nicotinic acid, and iron. A deficiency of calcium has been found in certain population groups while a vitamin D deficiency is quite prevalent among infants and children. It has been estimated that about 45,000,000 people or one-third of the population suffer to some extent from malnutrition. This situation has resulted from poor food habits, the wide use of refined foods, and financial inability of low-income groups to purchase "protective foods" such as milk, butter, eggs, meat, and vegetables.

---

[108] George Garnatz, Kroger Food Foundation, Cincinnati, Ohio, private communication.

Ever since the time of the Reverend Sylvester Graham (1794–1851), the vigorous advocate of whole wheat meal, white flour has been criticized as a food; within the past few years scientific observations have provided an increasing measure of support for these criticisms. The milling of wheat to refined white flour removes a large portion of the vitamins and minerals. Since, on the average, about $6^1/_2$ oz. of flour are consumed per person daily in the United States, a quantity which supplies about one-fourth of the normal energy requirements, the refinement of wheat results in a considerable reduction in the intake of the B complex vitamins, calcium and iron.[104]

Many agencies have advocated a return to whole wheat or graham flour or, alternatively, the milling of long-extraction flours in which more of the vitamins and minerals of the wheat would be retained. In several countries efforts have been made at various times to increase the consumption of whole wheat flour but they have been quite generally unsuccessful. Food selection by the consuming public is based on appeal to the eye and to taste rather than on nutritional aspects. In the United States, less than 3% of the flour consumed is whole wheat flour and it was realized by United States millers, bakers, and many nutrition authorities that a rapid improvement in general nutrition could not be hoped for by attempting to further the consumption of long-extraction or whole wheat flours.

During recent years several of the vitamins have become commercially available in pure form at reasonable cost and chemical and microbiological methods of vitamin assay have been developed. It was therefore possible to make controlled additions of both vitamins and minerals to the present types of refined food products which have long met with general public acceptance. Accordingly, in 1940, the Millers National Federation suggested that the new flour standards to be promulgated under the Federal Food, Drug, and Cosmetic Act of 1938 should include provision for the addition of vitamins and minerals to wheat flour.

The formulation of these standards proved to be a difficult problem. Consideration had to be given to the necessity for adding any particular vitamin or mineral and also to whether the additions of specific vitamins and minerals were to be made on the basis of restoring the quantities lost in processing or, alternatively, whether they should be based on the deficiencies of the various nutritional factors in the diets of the population in general and the place occupied by wheat flour in such diets. Several, including the wheat milling industry, considered that the flour standards should logically be based on the *restoration* of nutritionally important vitamins and minerals removed in the milling process. On the other hand, because of its prominent place in the human diet, many nutritionists regarded wheat flour as a logical medium by which the general nutrition of the population could be improved; that is, they favored the principle of *fortification*.

---

[104] C. G. Ferrari, *Northwestern Miller*, Almanac, No. 5, 210 (5), 3 (April 29, 1942).

After extended hearings, the proposed standards for flour and other wheat mill products, including provision for the enrichment of flours and farina, were published in May, 1941,[105] and became effective January 1, 1942. The technical terms "restoration" and "fortification" were replaced by the legal term "enrichment" and *niacin* was adopted as the official and proper name for nicotinic acid in order to avoid confusion with nicotine on the part of the layman.

The flour standards in regard to enriching ingredients were as follows:

Ingredient	Content per pound	
	Min.	Max.
Required ingredients:		
Thiamine, mg.	1.66	2.5
Riboflavin, mg.	1.2	1.8
Niacin (nicotinic acid or nicotinic acid amide), mg.	6.0	24.0
Iron, mg.	6.0	24.0
Optional ingredients:		
Calcium, mg.	500	2000
Vitamin D, U. S. P. units	250	1000
Wheat germ (or partly defatted wheat germ)	Not more than 5%	

Because of insufficient supplies, the riboflavin requirement was repeatedly postponed and never became effective under these original standards.

Subsequent to the hearings upon which these standards were based, hearings were held on the bread standards, and the proposed regulations for bread were incompatible with those in effect for flour. New information on human nutritional requirements and on the vitamin and mineral composition of the diets of various income groups indicated that the consumption of enriched flours complying with the present requirements would not result in the nutritional improvement which was originally expected. Thus, the estimates of the amount of niacin required in the flour to prevent the incidence of pellagra were too low; also, the amount of iron needed to give the most beneficial results was underestimated. Improved methods of niacin assay showed that unenriched white flour contains about 5 to 6 mg. of niacin per lb. as compared with 20 to 30 mg. per lb. in whole wheat. The wide range between the minimum and maximum levels for the different ingredients would lead to varying degrees of enrichment and the confusion of the consumer through competitive advertising claims. The milling industry was of the opinion that the standards should provide for nutritionally established minimum levels with reasonable tolerances above these levels to insure practical attainment of the standards. The question also arose as to whether calcium should be made a required rather than an optional ingredient.

These considerations led to a rehearing on the flour standards in the spring of 1943. As a result, the revised standards given in the succeeding section were promulgated and became effective October 1, 1943. Adequate supplies of riboflavin made it possible to place the riboflavin requirement in effect coincident with the other provisions of the new definitions.

---

[105] *Federal Register*, 6 (63), 1734 (1941).

On the basis of the tentative flour standards originally proposed, enrichment of flour and of bread was actually begun in February, 1941. By the end of 1942, it was estimated that about 65% of all family flour (sold to the retail trade) was enriched; by the summer of 1943, the estimate had risen to 80%. This new departure in food processing has therefore made rapid strides.

In the United States, long-extraction flours are manufactured to some extent, but nearly all the enriched flour is prepared by the addition of the enriching ingredients to the usual grades of refined white flour. In several other countries, however, the production of long-extraction flours has been encouraged as a means of improving the general nutrition of the population. In Canada, the addition of synthetic vitamins or minerals to flour has been prohibited by law and, on the recommendation of Canadian nutritionists, the Department of Health established standards, effective March 31, 1942, for "Vitamin B White Flour (Canada Approved)." As amended in August, 1943, the standard provides that flours marketed under this designation must comprise at least 70% of the wheat from which it was milled and must contain, on a moisture-free basis, not more than 0.70% ash and have a natural thiamine content of not less than 2.65 γ per g. (1.2 mg. per lb.). Such a flour normally represents from about 77 to 80% of the wheat kernel, depending upon the thiamine content of the wheat and the method of manufacture. It is naturally darker in color and somewhat inferior in general baking properties as compared with the standard grades.

In 1940, the British Ministry of Food announced that all white flour in Great Britain would be enriched with thiamine as soon as adequate supplies of the vitamin could be produced. Since May, 1941, all Canadian flour shipped to Britain has contained slightly more than 70 mg. of added thiamine per 100 lbs. of flour (total thiamine content is about 3 γ per g.). Also, British millers were required to produce, in addition to their regular grades, "National Wheat Meal," representing 85% of the wheat kernel. This product contains between 3 and 4 γ of thiamine per g. but has not met with ready consumer acceptance. As a war measure, the British Government has recently required English millers to produce only National Wheat Meal; the addition of calcium carbonate (*creta praeparata*) at the rate of 7 oz. per sack of 280 lbs. is required.

American nutritional authorities recognize that, although additions of synthetic vitamins and minerals to correct deficiencies as a result of food processing do not constitute a perfect solution of the nutritional problem, they do represent an important step forward. Aside from any consideration of the relative nutritive merits of enriched and long-extraction flours, a general improvement in nutrition is contingent upon the widespread consumption of the flours in question. Nutritional education may eventually overcome long-established dietary habits and preferences based on subjective factors, but the history of such attempts in the past implies that any such voluntary change will be a slow and gradual process.

For a more adequate treatment of the nutritive properties of flour and of the background of vitamin and mineral enrichment, the reader is referred to the publications of Bing,[106] Cowgill,[107] Sherwood,[108] Sherman and Pearson,[109] Taylor,[110] Wilder,[111] and Williams.[112]

(*h*) *Vitamin and Mineral Requirements of Enriched Flours*

The present standards in regard to enriching ingredients are given in Table 140.

The vitamins may be added in a harmless carrier which must be used only in the quantity necessary to effect an intimate and uniform admixture of the vitamins with the flour. Iron and calcium can be added only in forms which are harmless and assimilable.

TABLE 140

STANDARDS FOR ENRICHED FLOUR, EFFECTIVE OCT. 1, 1943, UNDER THE FEDERAL FOOD, DRUG, AND COSMETIC ACT OF 1938[a]

Ingredient	Content per pound	
	Min.	Max.
Required ingredients:		
Thiamine, mg.	2.0	2.5
Riboflavin, mg.	1.2	1.5
Niacin or niacin amide, mg.	16.0	20.0
Iron, mg.	13.0	16.5
Optional ingredients:		
Calcium, mg.	500	625
Vitamin D, U. S. P. units	250	1000
Wheat germ (or partly defatted wheat germ)	Not more than 5%	

[a] *Federal Register*, 6 (63), 1734 (1941); 8 (131), 9115 (1943). These standards apply to the enriching ingredients for enriched flour and enriched bromated flour. The enriching ingredients for enriched self-rising flour are as given above with the exception that the maximum calcium content is 1500 mg. per lb.

The minimum requirements for enriched flour, in comparison with the composition of patent, long-extraction, and whole wheat flours milled from hard red spring wheat, as compiled from data given by Ferrari,[113] are shown in Table 141. The daily contributions to the diet made by 6.5 oz. (the average daily flour consumption) of each of these flours are also shown; these are given as percentages of the daily allowances which were recommended at the rehearing on the flour standards. The fact that wheat is a minor source of riboflavin was taken into consideration in setting the standards, since the minimum riboflavin requirement exceeds the whole wheat level.

---

[106] F. C. Bing, *J. Am. Med. Assoc.*, **113**, 593 (1939).

[107] G. R. Cowgill, *J. Am. Med. Assoc.*, **113**, 2146 (1939).

[108] R. C. Sherwood, "The Vitamin and Iron Content of Wheat and Flour and Some Aspects of Flour Enrichment," *Bull. Assoc. Operative Millers*, 1941, p. 1162.

[109] H. C. Sherman and C. S. Pearson, *Modern Bread from the Viewpoint of Nutrition.* Macmillan, New York, 1942.

[110] A. E. Taylor, *Wheat Studies, Food Research Inst.*, **18**, 77 (1941).

[111] R. M. Wilder, *Bakers' Tech. Digest*, **15**, No. 10, 180 (1941).

[112] R. R. Williams, *Ind. Eng. Chem.*, **33**, 718 (1941).

[113] C. G. Ferrari, *Northwestern Miller*, Almanac No. **210**, No. 5, 3 (April 29, 1942).

Aside from a slightly more yellow crumb color imparted by riboflavin, the required enriching ingredients have no measurable effect upon general baking properties. Flours of varying extraction, however, differ appreciably in this respect. Thus, bread made from long-extraction flours will be darker in crumb color and inferior in crumb grain and texture to patent flours; in baking such flours certain modifications in bakery procedures normally employed for shorter extraction flours are necessary in order to secure optimum results.

TABLE 141

COMPARATIVE VITAMIN AND IRON CONTENTS OF WHOLE WHEAT, PATENT, LONG-EXTRACTION, AND ENRICHED FLOURS AND THEIR RELATIVE CONTRIBUTIONS TO DAILY DIETARY REQUIREMENTS

Constituent	Composition in mg. per lb.				Recommended daily allowance, mg.[b]
	Whole wheat flour	Patent flour	Long-extraction flour[a]	Enriched flour minimum[b]	
Thiamine	2.20	0.35	1.2	2.0	1.6
Riboflavin	0.50	0.15	0.2	1.2	2.3
Niacin	25	3.5	9.4	16.0	16.0
Iron	18	3.0	9.0	13.0	12.0

Approximate Percentages of Daily Requirements Supplied by 6.5 Oz. Various Flours

Constituent	Whole wheat flour	Patent flour	Long-extraction flour	Enriched flou minimum
Thiamine	56	9	30	51
Riboflavin	9	3	3	21
Niacin	64	9	24	41
Iron	61	10	30	44

[a] Flour representing approximately 80% of the wheat kernel, as manufactured from western Canadian hard spring wheat to meet the specifications for "Vitamin B White Flour (Canada Approved)."

[b] From *Federal Register*, 8 (131), 9115 (1943).

Addition of vitamins and minerals to cereal breakfast foods other than flour and farina may be made at the discretion of the manufacturer, and have been encouraged by the Food and Nutrition Board of the National Research Council as well as by the Council on Foods of the American Medical Association.

(*i*) *Definitions and Standards for Common and Club Wheat Food Products*

Definitions and standards of identity for flour and other wheat mill products, effective January 1, 1942, have been established under the Federal Food, Drug, and Cosmetic Act of 1938.[114] Those which are summarized below are all defined as prepared from "cleaned wheat other than durum wheat and red durum wheat" and in every case the moisture content must not exceed 15% as determined by the official A. O. A. C. vacuum oven method.[115]

---

[114] *Federal Register*, 6 (63), 1734 (1941).

Crushed wheat, coarse ground wheat is a whole wheat product crushed to such fineness that 40% or more will pass through a No. 8 sieve but less than 20% will pass through a No. 20 sieve.

Cracked wheat is made by cracking or cutting the entire wheat into angular fragments of such size that 90% will pass through a No. 8 sieve but not more than 20% will pass through a No. 20 sieve.

Farina represents coarsely ground endosperm particles, sufficiently freed from bran and germ to give an ash content not exceeding 0.6% on a dry matter basis. The granulation is such that it will completely pass through a No. 20 sieve but not more than 3% will pass through a No. 100 sieve.

Whole wheat flour, graham flour, entire wheat flour is a whole wheat product ground so that at least 90% will pass through a No. 8 and 50% through a No. 20 sieve. Malted wheat, malted wheat flour (not exceeding 0.5%), and malted barley flour (not exceeding 0.25%) may be used singly or in combination to compensate for any natural deficiency of enzymes. Provision is made for bleaching with nitrogen chloride, chlorine, or a mixture of chlorine and nitrosyl chloride; if a bleaching agent is used the product must be labeled "bleached."

Bromated whole wheat flour conforms to the above definition for whole wheat flour, except that it contains potassium bromate in a quantity not exceeding 75 p. p. m.

Flour, white flour, wheat flour, plain flour is a ground bolted wheat product which is freed from bran and germ to such an extent that the ash content does not exceed 0.05% of the protein plus 0.35; these calculations are made on a moisture-free basis. One of the cloths through which flour is bolted has openings not larger than those of woven-wire cloth designated "149 micron (No. 100)," Table 1 of "Standard Specifications for Sieves" (U. S. Dept. of Commerce, National Bureau of Standards L. C. 584, March 1, 1940). Provision is made for bleaching and/or artificial aging of flour with one or more of (*1*) oxides of nitrogen, (*2*) chlorine, (*3*) nitrosyl chloride, (*4*) nitrogen trichloride, and (*5*) benzoyl peroxide in a carrier (1 part benzoyl peroxide with not more than 6 parts by weight of a mixture of either potassium alum or calcium sulfate and magnesium carbonate). If a bleaching agent is used, the flour must be labeled "bleached." The mill mix may contain malted wheat and malted wheat flour and/or malted barley flour may be added but the quantity of malted barley flour must not exceed 0.25%.

Bromated flour must conform to the standards for wheat flour above, and contain not less than 15% protein (moisture-free basis); potassium bromate is added in a quantity not exceeding 75 p. p. m. of the finished flour.

Phosphated flour, phosphated white flour, phosphated wheat flour must conform to the definition and standard for plain flour and contain between 0.25 and 0.75% of monocalcium phosphate.

Self-rising flour, self-rising white flour, self-rising wheat flour conforms to the definition and standard for plain flour to which salt, sodium bicarbonate, monocalcium phosphate, and/or sodium acid pyrophosphate have been added. Sufficient acid-reacting substance must be added to neutralize the sodium bicarbonate. The self-rising flour must contain enough of the leavening agents to evolve not less than 0.5% of its weight of carbon dioxide; the total weight of the leavening ingredients must not exceed 4.5 parts to each 100 parts of flour used.

Standards have also been established for **enriched bromated flour, enriched self-rising flour,** and **enriched farina.** Each of these conforms to the corresponding definitions for the nonenriched products except for their content of certain required and optional enriching ingredients. For enriched bromated flour, these are the same as for enriched plain flour (Table 140); the requirements for enriched self-rising flour differ only in the maximum limit for calcium, which is 1500 mg. Revised standards for enriched farina have not as yet been published (November, 1943).

---

[115] *Official and Tentative Methods of Analysis.* 5th ed., Assoc. of Official Agr. Chem., Washington, D. C., 1940.

(j) *Definitions of Wheat By-Product Feeds*

The following definitions for wheat by-product feeds have been approved by the Association of American Feed Control Officials.

Wheat Bran is the coarse outer covering of the wheat kernel as separated from cleaned and scoured wheat in the usual process of commercial milling.

Standard Middlings consists mostly of fine particles of wheat bran, wheat germ, and very little of the fibrous offal obtained from the "tail of the mill." This product must be obtained in the usual commercial process of milling, and must not contain more than 9.5% of crude fiber.

Flour Middlings consists of wheat standard middlings and wheat red dog combined in the proportions obtained in the usual process of milling, and must not contain more than 6% of crude fiber.

Gray Shorts (Gray Middlings or Total Shorts) consists of the fine particles of the outer bran, the inner or brewing bran, the germ, and the offal or fibrous materials obtained from the "tail of the mill." This product must be obtained in the usual process of commercial milling, and must not contain more than 6% of crude fiber.

Brown Shorts (Red Shorts) consists mostly of the fine particles of wheat bran, wheat germ, and very little of the fibrous offal obtained from the "tail of the mill." This product must be obtained in the usual commercial process of milling and must not contain more than 7.5% of crude fiber.

Wheat Bran and Standard Middlings consists of the two commodities as defined (see Wheat Bran and Wheat Standard Middlings), mixed in the proportions obtained in the usual process of commercial milling.

Wheat Low-Grade Feed Flour, a by-product obtained in the usual commercial process of flour milling, consists principally of wheat flour with small quantities of aleurone and fine wheat bran particles, and must not contain more than 1.5% of crude fiber.

Wheat Mixed Feed (Mill Run Wheat Feed) consists of wheat bran and the gray or total shorts combined in the proportions obtained in the usual process of commercial milling. This product must not contain more than 8.5% of crude fiber.

Wheat Red Dog, a by-product obtained in the usual commercial process of flour milling, consists principally of aleurone with small quantities of wheat flour and fine wheat bran particles, and must not contain more than 4% of crude fiber.

White Shorts or **White Middlings** consists of a small portion of the fine wheat bran particles and the wheat germ and a large portion of the fibrous offal obtained from the "tail of the mill." This product must be obtained in the usual process of flour milling and must not contain more than 3.5% of crude fiber.

Hard Wheat Mixed Feed (Mill Run Wheat Feed) consists of wheat bran and wheat flour middlings combined in the proportions obtained in the usual process of commercial milling. This product must not contain more than 9.5% of crude fiber.

3. Durum Wheat

(a) *Quality Characteristics of Durum Wheat*

Durum wheat is frequently called macaroni wheat because semolina, the coarsely ground, highly purified middlings obtained by milling durum wheat, is regarded as the premier raw material for the manufacture of macaroni. The majority of the macaroni-consuming public demands macaroni of an intense, clear yellow color; the products must be smooth on the surface and free from specks of any kind. Furthermore, high-quality macaroni is hard, brittle, translucent, and pliable; the long pieces should be capable of being bent to a considerable extent before breaking, with a clear glossy fracture. The real test of high-quality macaroni, however, is its behavior on boiling. When boiled for 10 min., a good macaroni will swell to at least twice its original size, but will retain its tubular shape and its firmness without becoming pasty. These requirements are most fully met when the raw material consists of the purified middlings and flour made from amber durum wheat. Red durums yield a visibly specky product possessing an unsatisfactory dull reddish-yellow or yellowish-brown color and are not in demand by the durum milling industry. Macaroni made from farina, the purified middlings milled from the hard types of common wheat, or common wheat flour products, is not so favorably received as that manufactured from durum semolina and flour. The macaroni fractures more easily, lacks translucency, is likely to be specky, possesses a dull chalky grayish-white color, and on cooking does not retain its shape as well; tending to yield a somewhat pasty mass which lacks the characteristic, somewhat sweet nutlike flavor of macaroni made from durum products.

Durum wheat differs in several particulars from the common or *vulgare* wheats. The kernel is considerably larger and more angular in form, the length often exceeding 10 mm. and the breadth 4 mm. As the name implies, the endosperm is extremely hard and vitreous, and contains a considerably higher concentration of carotenoid pigments than the varieties of hard red spring and hard red winter wheats considered satisfactory for the manufacture of bread flours. Analyses of American durum wheats, conducted by the United States Bureau of Chemistry, show the following range in composition: moisture, 8–12%; nitrogen, 1.77–3.10%; invert sugar, 0.17–1.20%; sucrose, 1.60–3.90%; fat, 1.10–2.40%; fiber, 1.70–2.85%; ash, 1.30–2.35%; phosphoric acid (P_2O_5), 0.70–1.20%; and weight per thousand kernels, 23.3–55.1 g.[116] These data indicate that, in general, durum wheat has a higher protein, lipid, ash, and sugar content than common wheat.

Many statements appear in the literature that the high protein content of durum wheat is an inherited characteristic, but the writer is of the opinion that the high protein content of durum wheats is more a result of their culture under semiarid conditions than of a genetic property. This is based on the results of a survey of the protein content of Western Canadian hard red spring and amber durum wheat from the same shipping points in three crop years; the mean protein content of durum wheat was actually 0.3% lower than that for hard red spring wheat.[117] The endosperm proteins of durum wheat, although apparently similar in chemical composition to those of common wheat, form gluten of different colloidal properties.[118] Durum flours yield a stiffer dough with less water than other wheat flours; this is a desirable characteristic for macaroni making since the water added to form the dough has to be removed after pressing it into the desired shape, but is undesirable from the point of view of the bread manufacturer, since it lowers the yield of bread per barrel of flour.

Investigations indicate that the physical properties of durum wheat starch differ from those of common wheat starch in having a greater swelling capacity upon gelatinization[119] and in being more susceptible to hydrolysis by the cereal amylases.[120] The latter property may well be due to a greater degree of mechanical injury of the starch granules in milling durum wheat as a result of the extremely vitreous nature of the endosperm.[121] Durum flour doughs show a marked tendency to "slacken," or become more mobile upon standing or fermentation. Until the past few years this characteristic of wheat flour doughs was attributed to excessive proteinase activity which resulted in a modification of the colloidal properties of the gluten proteins. In the instance of bread doughs, it seems fairly well established that the excessive slackening of doughs, such as occurs in the instance of flours made from sprouted wheat, is principally due to the liquefying action of α-amylase on wheat starch.[122] While there is some indication that durum wheat flours tend to be higher in proteolytic activity than common wheat flours, it seems reasonable to suppose that the excessive "softening" or slackening of durum flour doughs is associated with their high amylase activity.

---

[116] J. A. Le Clerc, *Cereal Chem.*, **10**, 383 (1933).

[117] W. F. Geddes, W. J. Eva, and N. Milton, *Sci. Agr.*, **20**, 599 (1940).

[118] R. H. Harris, *Cereal Chem.*, **14**, 695 (1937).

The marked difference in the macaroni and breadmaking processes coupled with the quality requirements of the American consumer imply that different criteria must be applied in the evaluation of wheat products for these two purposes. Macaroni making is a relatively simple physical process as compared with the manufacture of yeast-leavened products. The bread-eating public demands a loaf of relatively large volume possessing a creamy white crumb; in contrast, the critical French and Italian macaroni consumer requires a high yellow pigmentation in the product. Durum flours yield less bread per unit weight of flour and the resulting bread is usually lower in volume and possesses a decidedly more yellow crumb than common wheat flours. These properties are undesirable in an American bread flour; while durum wheats are used in Russia as bread flours, they do not meet American consumer requirements. In western Europe, durum wheats are frequently included in small percentages in bread wheat mixes, but are regarded as weak or filler wheats, in spite of their high protein content. The vitreous nature of durum wheat (which aids in the production of coarse granular particles rather than flour), the lower water absorption, and the higher pigment content of the endosperm are desirable for macaroni production. In general, therefore, hard *vulgare* wheats are regarded of high "quality" for the production of bread flours but of low "quality" for the production of granular material to be used in macaroni manufacturer; the reverse is true in the instance of durum wheat.

The quality of durum wheat for semolina milling is primarily associated with its ability to produce a high yield of semolina which is free of visible specks and which will produce macaroni of the desired brilliant yellow color. In normal years, little attention needs to be paid to protein content in the selection of durum wheats for milling purposes; however, in the manufacture of "long-goods" it is necessary to maintain the protein content above a more or less ill-defined minimum in order to avoid undue stretching during the drying process. Macaroni manufacturers prefer semolina containing 12% or more of protein (13.5% moisture basis) but in some crop years it is impossible for the millers to attain this level without sacrifice of color; normally, little difficulty is encountered unless the semolina protein drops below about 11%, if extra care is taken in the curing or drying process. There is some indication that macaroni processed from durum wheat of low protein content is somewhat deficient in translucency; on the other hand, under weather conditions which are conducive to abnormally high protein levels, the semolinas tend to produce macaroni of a less intense yellow color and possessing a dull reddish or brownish cast.[123] Le Clerc[124] states that a high-grade semolina contains 12% moisture, 11.5% protein, 1.8% lipids, and 0.6% ash.

---

[119] C. E. Mangels and C. H. Bailey, *Ind. Eng. Chem.*, **25**, 456 (1933).
[120] R. M. Sandstedt, M. J. Blish, D. K. Mecham, and C. E. Bode, *Cereal Chem.*, **14**, 17 (1937).
[121] R. M. Sandstedt, C. E. Jolitz, and M. J. Blish, *Cereal Chem.*, **16**, 780 (1939).
[122] F. C. Hildebrand and G. M. Burkert, *Cereal Chem.*, **19**, 27 (1942).

While durum millers have employed laboratory control for some years, it is only recently that systematic efforts have been made by cereal research laboratories to develop experimental milling, macaroni making and quality testing techniques for the evaluation of durum wheat quality. Since 1934, several papers have been published in this connection,[123–131] but much remains to be done before our techniques and knowledge of the factors affecting durum wheat quality will have attained the same status as those relative to wheat and flour quality for bread, biscuits, and pastry. At present, color is the most important quality index and it has been found that macaroni color is influenced by both environmental and varietal factors. Relatively cool seasons, with adequate rainfall, produce durum wheats of better color than hot, dry seasons.[132] In some years, the quality of durum wheat is adversely affected by fungi (*Helminthosporium* associated with other fungi), which develop initially at the germ end of the kernel when moisture conditions are favorable and cause a black or brownish discoloration of the seed coat; for this reason the condition is commonly called "black point" or "smudge." Durum wheat containing even small percentages of "black point" damage is shunned by the durum miller since the semolina produced from it tends to contain black specks.[133]

There is a wide variation in the relative quality of different varieties. Macaroni color cannot be predicted with any certainty from the visual appearance of the semolina or a determination of the quantity of yellow pigment; this is particularly true in the instance of the red durums, but certain varieties of amber durum, such as Golden Ball, of inherently high yellow pigment content, yield macaroni of an unsatisfactory dull brownish-yellow color.[134, 135] Of the varieties which are now widely grown commercially,[136] Mindum and Kubanka have the highest inherent quality; but durum wheat breeders are developing new varieties which may become prominent in future production.

---

[123] D. S. Binnington, and W. F. Geddes, *Cereal Chem.*, **13**, 497 (1936).

[124] J. A. Le Clerc, *Cereal Chem.*, **10**, 383 (1933).

[125] D. S. Binnington, and W. F. Geddes, *Can. J. Research*, **10**, 221 (1934).

[126] C. C. Fifield, *Cereal Chem.*, **11**, 330 (1934).

[127] D. S. Binnington, and W. F. Geddes, *Can. J. Research*, **10**, 221 (1934).

[128] D. S. Binnington, H. Johannson, and W. F. Geddes, *Cereal Chem.*, **16**, 149 (1939).

[129] C. C. Fifield, G. S. Smith, and J. F. Hayes, *Cereal Chem.*, **14**, 661 (1937).

[130] R. L. Cunningham and J. A. Anderson, *Cereal Chem.*, **20**, 171, 482 (1943).

[131] R. H. Harris, and L. D. Sibbitt, *Cereal Chem.*, **19**, 388 (1942).

[132] C. E. Mangels, *Cereal Chem.*, **9**, 485 (1932).

[133] R. H. Harris, and L. D. Sibbitt, *Cereal Chem.*, **19**, 403 (1942).

Le Clerc[137] and Alsberg[138] have reviewed the production, properties, and utilization of durum wheat and its products; these references should be consulted for more detailed information.

(b) *Definitions and Standards for Durum Wheat Products*

Under the new Federal Food, Drug, and Cosmetic Act of 1938 which became effective June 25, 1939, Standards of Identity, effective January 1, 1942, were established for semolina, durum flour, and whole durum wheat flour.[139] Semolina and durum flour must be freed from bran and germ to such an extent that their ash contents do not exceed 1.5% and 0.92%, respectively, calculated to a moisture-free basis. Semolina must be of such a granulation that it will pass through a No. 20 sieve but not more than 3% will pass through a No. 100 sieve, whereas durum flour will pass bolting cloth which has openings of similar dimensions to that of the No. 100 wire sieve.[140] Durum wheat flour is subject to the same requirements as whole wheat flour (see page 668), except that it is prepared from durum or red durum wheat. The moisture content of semolina, durum flour, and whole durum wheat flour must not exceed 15%.

4. Macaroni Products (Alimentary Pastes)

(a) *Definition*

Macaroni products consist basically of dough, made with one or more of semolina, farina, or flour and water, which has been formed under pressure into a variety of sizes and shapes and then dried. This entire group of foods is usually termed "alimentary pastes" (which is a translation of the French term *pâtes alimentaries* and the Italian *paste alimentari*), and embraces macaroni itself, spaghetti, noodles, vermicelli, alphabets, rings, stars, sea shells, and many other special forms.

(b) *Production and Consumption*

The commercial manufacture of macaroni in the United States was started about 90 years ago but the industry has made its most rapid growth since 1900, its development more or less paralleling the establishment and expansion of the domestic production of durum wheat. Just prior to the first World War, macaroni imports (chiefly from

---

[134] D. S. Binnington, and W. F. Geddes, *Cereal Chem.*, **14**, 293 (1937).

[135] D. S. Binnington, and W. F. Geddes, *Cereal Chem.*, **16**, 384 (1939).

[136] J. A. Clark, and K. S. Quisenberry, U. S. Dept. Agr., *Circ.* **634** (1942).

[137] J. A. Le Clerc, *Cereal Chem.*, **10**, 383 (1933).

[138] C. L. Alsberg, *Wheat Studies, Food Research Inst.*, **15**, 337 (1939).

[139] *Federal Register*, **6** (63), 1734 (1941).

[140] See "Standard Specifications for Sieves," U. S. Dept. Agr., *Lab. Circ.* **584** (Mar. 1, 1940).

Italy) amounted to nearly 130,000,000 lbs., or 40% of the domestic production. After 1920, the United States became an appreciable exporter and the imports during the past decade have amounted to only about 0.5% of the domestic production.[141] In 1939, according to the U. S. Census Report, there were 328 establishments making macaroni products. Statistics showing the quantity and value of the principal materials consumed and produced are summarized in Table 142.

TABLE 142

QUANTITY AND VALUE OF PRINCIPAL MATERIALS CONSUMED, AND MACARONI PRODUCTS MANUFACTURED, BY THE MACARONI AND OTHER INDUSTRIES IN 1939[a]

Principal Materials Consumed

Product	Quantity	Cost
Semolina, bbl.	2,159,987	$11,173,419
Flour, durum, bbl.	816,755	3,760,088
Flour, other, bbl.	508,165	2,136,832
Sugar, sucrose, lbs.	501,550	24,416
Sugar, corn, lbs.	89,479	3,526
Eggs	......	2,603,183

Macaroni Products Manufactured

Product	Quantity, lbs.	Value
Macaroni, spaghetti, and vermicelli	572,023,610	$35,182,258
Noodles, egg and other egg products	101,601,228	9,955,026
Noodles, plain or water	10,765,410	729,958
All products	684,390,248	$45,867,242

[a] Compiled from U. S. Census Report for 1939. The present definitions and standards for noodles require that all noodles must contain egg solids.

Macaroni plants are located in many states, but the production is particularly heavy in the Atlantic States, where New York, Philadelphia, and Boston are the principal centers. Illinois and California are also important producers.

(c) *Types of Macaroni Products*

There are many types of macaroni products which differ from each other in size or shape. They include smooth and corrugated tubular forms, solid cylindrical forms, and flat ribbons of various sizes, as well as numerous special shapes, such as crosses, stars, and alphabets. The smooth, tubular forms in various diameters are known as macaroni; of the solid-rod forms, vermicelli and spaghetti are the most common. Noodles (which contain egg solids) are the best known of the ribbon types. The more common types are illustrated in Fig. 58.

From the standpoint of manufacture, the various macaroni products are classified as "long-goods" and "short-goods"; the long-goods are dried over sticks while the short-goods are dried on screen trays. For the production of long-goods, such as macaroni, spaghetti, and vermicelli, semolina has long been regarded as the premier raw material in order to avoid manufacturing troubles from undue stretching and breaking during the drying or curing process. For "short-goods," such as alphabets, elbows, bow knots, and shells, the use of semolina is not so important, since these products are dried on trays. For manufacturing noodles, durum or common wheat flour is invariably used; the egg proteins act as a binder and impart a pleasing yellow color to the finished product. Long-goods are made not only with semolina but also frequently with mixtures of semolina and farina or farina alone; in recent years there has been an increasing use of flour by the macaroni industry. Plants on the Pacific coast use large quantities of farina and flour milled from white wheat; in Kansas,

---

[141] J A. Le Clerc, *Cereal Chem.*, 10, 383 (1933).

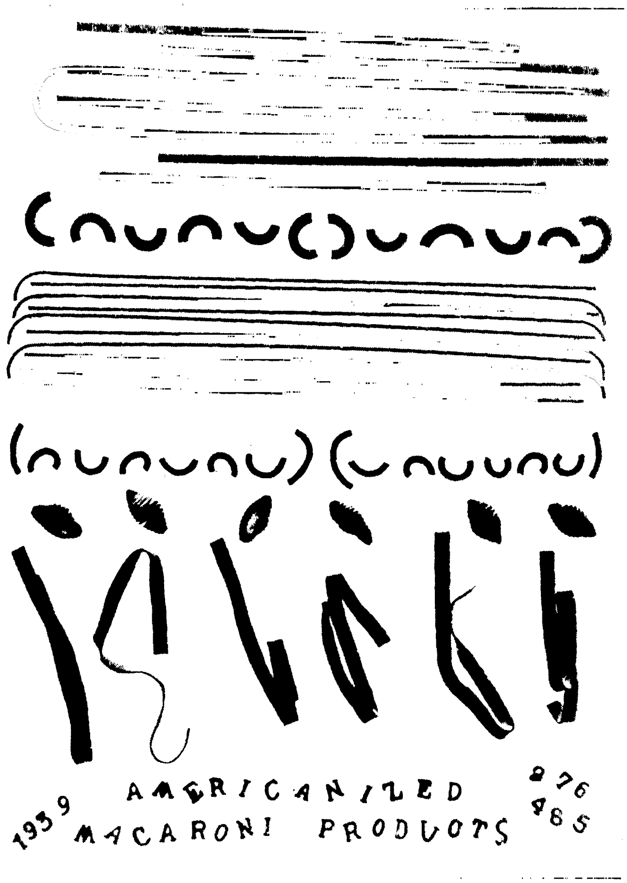


Fig 58 –Common types of macaroni products Reading from top to bottom, the shapes are called: macaroni, macaroni elbows, spaghetti, spaghetti elbows, shells, egg noodles, alphabets, and numerals. (Reproduced by courtesy of the National Macaroni Institute, Braidwood, Illinois.)

farina and flour made from hard winter wheats are widely used. In the manufacture of macaroni products for the French and Italian trades, however, consumer preference requires the use of durum semolina or flour.

(*d*) *Definitions and Standards for Macaroni Products*

Standards for macaroni products under the Federal Food, Drug, and Cosmetic Act (effective June 25, 1939) have not been promulgated (January, 1944) but the findings of fact and the proposed standards have been published.[142]

Macaroni products are defined as the class of food prepared from one or more of semolina, durum flour, farina, or flour with or without the addition of certain optional ingredients some of which, if used, must be kept within certain defined percentage ranges. These optional ingredients are: (*a*) egg white, frozen egg white, or dried egg white, from 0.5 to 1.0% solids basis (the purpose of the egg white is to prevent the collapse of thin-walled macaroni products during and subsequent to cooking); (*b*) disodium phosphate, from 0.5 to 1.0% (this is added to accelerate the cooking); (*c*) onions, celery, garlic, bay leaf (one or more in sufficient quantity to season); and (*d*) salt (in sufficient quantity to season). **Macaroni** is the macaroni product which consists of tube-shaped units from 0.06 to 0.27 in. in diameter; **spaghetti** comprises tube-shaped or cord-shaped units from 0.06 to 0.11 in. in diameter, while **vermicelli** consists of cord-shaped units not more than 0.06 in. in diameter. When the optional ingredients are used they must be specified.

In addition to these definitions for "macaroni products," standards have also been proposed for **milk macaroni products, whole wheat macaroni products, wheat and soy macaroni products,** and **vegetable macaroni products,** each of which may be produced in shapes and sizes corresponding to the definitions for macaroni, vermicelli, and spaghetti. None of these types is permitted to contain egg white or disodium phosphate as optional ingredients but may contain the seasoning agents specified under (*c*) and (*d*) above.

Milk macaroni products must contain not less than 3.8% milk solids; the milk ingredients may be supplied in the form of milk (which is then the sole moistening ingredient used in preparing the dough) or concentrated milk, evaporated milk, dried milk, or a mixture of butter with one or more of skim milk, concentrated skim milk, evaporated skim milk in such proportions that the weight of non-fat milk solids is not more than 2.275 times the weight of milk fat.

In whole wheat macaroni products, durum whole wheat flour and/or whole wheat flour comprise the sole wheat ingredient. **Wheat and soy macaroni products** have the same wheat ingredients as **macaroni products** but must contain not less than 15% soy flour. **Vegetable macaroni products** contain not less than 3% solids derived from tomato (red varieties), artichoke, beet, carrot, parsley, or spinach; these may be used in fresh, canned, dried, or puree form.

Noodle products are defined as the class of food prepared from dough containing one or more of semolina, durum flour, farina, or flour and not less than 5.5% of the solids of egg or egg yolk; with or without one or more of onions, celery, garlic, bay leaf, and salt. Under noodle products definitions are given for **noodles, egg noodles** (ribbon-shaped units), as well as for **egg macaroni, egg spaghetti,** and **egg vermicelli,** which conform to the shapes and dimensions previously given under "macaroni products." Standards for **wheat and soy noodle products,** and **vegetable noodle products** are also proposed; these are similar to the corresponding macaroni products, except, of course, that they must contain at least 5.5% of egg solids.

Each paste product must be named on the label in a manner which conforms with its character and shape as such as, for example, "macaroni," "milk spaghetti," "tomato vermicelli," "wheat and soy noodles," "wheat and soy egg spaghetti," "tomato egg vermicelli," etc. All the forms of alimentary pastes must contain not less than 87% solids.

---

[142] *Federal Register*, 7 (249), 10728 (1942).

It is important to note that no standards providing for the enrichment of alimentary pastes with vitamins and minerals or for the inclusion of yeast or wheat germ (as such) have been proposed. In the "findings of fact" it is pointed out that alimentary pastes are not effective vehicles for the distribution of vitamins and minerals to any large segment of the population. The per capita consumption is low and, as most of the pastes are cooked in relatively large quantities of water which is discarded, there is a large economic waste inherent in the addition of water-soluble vitamins to such products. Government authorities point out that the enrichment of foods must be restricted by regulation, otherwise the consumer will be misled as to his nutritional needs by "claims of enhanced nutritional value for a multiplicity of indiscriminately enriched foods."

X. CEREAL PREPARATIONS[143]

1. History and Production

A great variety of cereal preparations are available on the American market. These consist principally of breakfast foods, of which there are two major types: those which require cooking before serving, and those which are cooked and ready to serve.

TABLE 143

PRODUCTION AND VALUE OF CEREAL PREPARATIONS IN THE UNITED STATES IN 1939[a]

Product	Production			Value		
	Ready to serve, lbs.	To be cooked, lbs.	Total, lbs.	Ready to serve	To be cooked	Total
Wheat	299,539,205	153,452,588	452,991,793	$37,665,239	$10,463,153	$ 48,128,392
Oats	[b]	[b]	529,672,399	[b]	[b]	24,832,576
Corn	[b]	[b]	250,289,091	[b]	[b]	21,240,052
Oats and corn	231,095,091	548,866,399	779,961,490	20,637,042	25,435,586	46,072,628
Other grains and mixtures of grains	45,859,023	9,803,532	55,662,555	8,762,800	507,769	9,270,569
Total breakfast foods	576,493,319	712,122,519	1,288,615,838	$67,065,081	$36,406,508	$103,471,589
Corn grits and hominy for human consumption	[b]	[b]	227,477,645	[b]	[b]	3,520,260
Other cereal preparations, including coffee substitutes	....	....	....	....	....	4,985,881
Total value						$111,977,730

[a] Compiled from U. S. Census Report on cereal preparations.
[b] Figures withheld to avoid disclosing data reported by individual establishments.

The raw cereal breakfast foods include products made from all the common cereals and mixtures of different cereals such as corn meal, rolled oats, cracked wheat, and farina, as shown.

The conception of ready-prepared cereals as articles of commerce and their general manufacture and distribution on a national basis occurred from about 1895 to 1905. Today, the manufacture of ready-to-serve breakfast cereals and cereal coffee sub-

---

[143] The author is indebted to C. G. Ferrari, General Mills, Inc., Minneapolis, Minn.; S. N. Holiday, National Biscuit Co., New York; A. G. Olsen, General Foods Corp., Hoboken, N. J.; and M. R. Warren, The Quaker Oats Co., Cedar Rapids, Ia., for their courtesy in supplying material for this section.

TABLE 144

PROXIMATE CHEMICAL COMPOSITION AND FUEL VALUE OF CEREAL BREAKFAST FOODS[a]

Material	Moisture, %	Ash, %	Protein, %	Fiber, %	Carbohydrate other than fiber, %	Fat, %	Cal. per 100 g.
Corn preparations:							
Collins' Golden Purity Hulled Corn	12.2	1.2	7.9	0.6	76.1	2.0	354
Corn Kix	7.6	3.2	8.0	0.5	78.8	1.9	365
Kellogg's Corn Flakes	4.3	3.1	8.0	0.4	83.9	0.3	370
Post Toasties Corn Flakes	6.3	3.0	8.0	0.4	82.1	0.2	362
Quaker Hominy Grits	13.0	0.6	7.7	0.4	77.5	0.8	348
Sun Seal Sunnycorn	12.1	0.4	8.4	0.4	78.2	0.5	351
Oat preparations:							
Coarse American Oatmeal	10.4	2.0	15.8	1.0	65.2	5.6	375
H-O Hornsby's Oats	10.5	2.0	16.0	1.2	64.3	6.0	365
Irish Oatmeal	6.7	1.9	15.3	1.1	67.8	7.2	398
Irish Oat Meal	9.3	2.0	15.3	0.9	65.5	7.0	387
Quaker Rolled White Oats	10.7	2.1	16.6	1.4	63.2	6.0	374
Rolled Oats	10.5	1.8	17.4	1.2	62.8	6.3	378
Scotch Oatmeal	10.7	2.2	11.6	1.6	67.5	6.4	374
Tremont Brand Regular Rolled Oats	10.5	1.9	15.1	1.1	64.9	6.5	379
Rice preparations:							
B. C. Rice Flakes	9.5	3.3	6.3	0.5	80.1	0.3	349
Cream of Rice	11.8	0.5	7.4	0.3	79.6	0.4	352
Heinz Rice Flakes	6.8	3.0	7.5	4.2	78.1	0.4	346
Quaker Brand Puffed Rice	9.0	0.4	6.0	0.4	83.9	0.3	363
Rice Krispies	5.4	3.3	6.8	0.3	83.9	0.3	366
Rye preparations:							
Cream of Rye	11.3	1.8	11.1	1.3	72.4	2.1	353
Wheat preparations:							
B-C Vita Wheat	8.8	1.0	10.9	0.7	76.7	1.9	368
Cooked Wheat	3.4	4.3	10.5	2.6	76.8	2.4[c]	371
Cracked Wheat	12.7	1.7	9.6	1.9	72.1	2.0	345
Cream of Wheat	11.6	0.4	12.6	0.2	74.5	0.7	355
Enright's All O'The Wheat Old Fashioned Cereal	12.0	1.5	15.0	2.4	66.7	2.4	349
Force-Toasted Whole Wheat Flakes	5.7	3.2	9.3	1.1	78.5	2.2[c]	371
Hecker's Cream Farina	11.3	0.4	12.3	0.2	74.9	0.9	357
Huskies Whole Wheat Flakes	9.1	3.4	9.6	1.7	75.1	1.1	349
Kellogg's Whole Wheat Biscuit	9.7	2.1	10.3	2.5	73.7	1.7[c]	351
Kellogg's Wheat Krispies	6.5	4.3	8.8	1.8	77.4	1.2	356
Kellogg's Whole Wheat Krumbles	4.8	3.3	9.4	2.4	78.0	2.1[c]	369
Muffets—Whole Wheat Biscuit	10.4	1.6	10.3	1.7	74.7	1.3	352
National Biscuit Shredded Biscuit	10.4	1.6	9.8	1.8	75.1	1.3	352
Overland Wheat Cereal	11.8	0.4	12.0	0.3	74.8	0.7	354
Pettijohn's Rolled Wheat, with all the bran	12.3	1.7	9.9	2.2	72.0	1.9	345
Pillsbury's Farina	11.7	0.4	11.8	0.2	75.2	0.7	354
Post-O The New Wheat Cereal	10.9	3.8	11.6	2.0	70.5	1.2	339
Quaker Brand Puffed Wheat	7.9	1.6	15.5	2.0	71.3	1.7	363
Quaker Farina	10.9	0.5	11.4	0.3	76.1	0.8	357
Quaker Wheat Crackels	5.8	5.5	11.8	0.7	75.0	1.2[c]	358
Ralston Wheat Cereal	10.8	1.7	16.6	1.1	67.6	2.2	357

TABLE 144 (*Continued*)

PROXIMATE CHEMICAL COMPOSITION AND FUEL VALUE OF CEREAL BREAKFAST FOODS[a]

Material	Moisture, %	Ash, %	Protein, %	Fiber, %	Carbohydrate other than fiber, %	Fat, %	Cal. per 100 g.
Shredded Ralston Toasted Whole Wheat	6.0	4.1	9.9	2.0	75.7	2.3[c]	364
Sunshine Rippled Wheat Cereal Biscuit	11.1	3.9	9.5	2.0	71.8	1.7[c]	341
Wheatena	1.9	1.5	11.8	1.5	73.7	2.4	346
Wheaties	7.1	4.7	13.2	1.4	72.5	1.1	353
Wheatsworth Whole Wheat Cereal	9.1	1.7	14.0	2.4	70.8	2.0	357
Miscellaneous preparations:[b]							
B-C Battle Creek "ZO"	7.0	3.3	15.6	1.6	71.7	0.8	356
B-C Cooked Bran	7.9	6.9	16.4	9.3	54.8	4.7[c]	328
B-C Fig and Bran Flakes	8.4	4.2	9.5	2.4	73.7	1.8	349
B-C Fig and Bran	6.6	7.6	11.5	6.7	65.1	2.5	329
B-C Krusty Bran	7.1	6.1	11.4	6.5	66.4	2.5[c]	334
B-C Lima Bean-Rice Flakes	10.9	3.2	13.0	2.5	68.9	1.5	341
B-C Toasted Gluten Bran Flakes	9.5	3.2	48.8	6.9	25.4	6.2[c]	353
B-C Washed Bran	9.4	5.4	16.9	13.4	51.9	3.0	303
Cellu Breakfast Crisp	7.4	4.1	4.5	21.5	40.1	22.4[d]	179[e]
Ceremel—A Laxative Food	8.7	5.3	18.3	6.4	54.8	6.5	351
Cerevim	10.0	3.4	19.3	1.7	63.4	2.2	425
Grape Nuts Bran Flakes	6.5	3.0	10.5	2.0	75.3	2.7[c]	368
Grape Nuts	5.1	2.6	11.3	1.7	77.1	2.2[c]	374
Jackson Meal	10.8	2.5	15.3	3.3	65.6	2.5	347
Kellogg's All-Bran	4.3	8.8	13.0	8.7	62.7	2.5	326
Kellogg's 40% Bran Flakes	5.6	4.9	10.4	3.8	73.5	1.8	352
Lister's Starchless Breakfast Food	10.7	2.3	5.9	6.8	72.9	1.4	328
Maltex Cereal	9.6	1.5	15.3	1.2	70.9	1.5	358
Maypl Cereal	9.3	1.9	15.6	0.9	67.9	4.4	374
Pep	4.0	4.0	10.6	3.1	76.5	1.8	365
Post's 40% Bran Flakes	7.5	4.4	12.3	3.5	70.5	1.8	348
Roman Meal	10.7	5.3	15.1	4.4	61.8	2.7	332
Uncle Sam Laxative Breakfast Food	7.8	2.8	18.5	2.8	56.7	11.4	403
Weetabix	8.6	2.4	9.4	2.2	75.0	2.4[c]	356
Wheat Oata—Wheat and Oat Porridge	10.7	1.9	15.6	1.4	65.9	4.5	369

[a] E. M. Bailey, Conn. Agr. Expt. Sta., *Bull.* **437**, 1940.

[b] Miscellaneous preparations generally consist of a cereal base, supplemented with fruit, vegetable gum, or bran to produce laxative effects.

[c] Baked product method.

[d] Largely mineral oil.

[e] "Fat" not included.

stitutes is an important industry. As shown in Table 143, over 1¼ billion lbs. of breakfast cereals were produced in 1939 with a total value in excess of $103,000,000. Of the total production 44.74% (576,493,319 lbs.) comprised ready-to-serve cereals; these represented 64.81% of the total value. In addition, coffee substitutes and other cereal preparations valued at nearly $5,000,000 were produced. As there are so many of these foods on the market it is only possible, here, to describe the preparation of a few which have been selected to represent general types, in so far as manufacturing operations are concerned.

The manufacture of raw and of ready-to-serve breakfast cereals is described in Volume II, Chapter XV.

2. Chemical Composition of Cereal Breakfast Foods

The proximate compositions of several breakfast foods, including both ready-to-serve and to-be-cooked-before-serving types, and of a cereal coffee substitute are given in Tables 144 and 145.

TABLE 145

PROXIMATE COMPOSITION OF POSTUM CEREAL AND INSTANT POSTUM[a]

Material	Moisture, %	Protein, %	Fat, %	Fiber, %	Ash, %	N-free extract (by diff.), %
Postum Cereal	5.0	12.9	2.4	10.7	5.5	63.5
Instant Postum	2.4	6.4	0.2	0.0	7.8	83.2[b]

[a] Courtesy of A. G. Olsen, General Foods Corp., Hoboken, N. J.

[b] The nitrogen-free extract of Instant Postum comprises: dextrin, 62.7%; sucrose, 7.4%; invert sugar, 6.3%; and other carbohydrates, 6.8%.

XI. COTTONSEED, PEANUT, AND SOYBEAN FLOURS[144]

1. History and Production

Although this chapter is concerned with the cereal flours, it is convenient to consider here the production and properties of edible flours from the cottonseed, peanut, and soybean.

The cotton plant, of which there are many species, belongs to the mallow family (*Malvaceae*) and the genus, *Gossypium*, while the peanut (*Arachis hypogaea*) and the soybean (*Soja max*) are legumes (*Leguminosaea*).

The development of acceptable flours from these sources is so very recent that, as yet, they are used only to a rather limited extent in the American diet. However, the present interest in fortified foods, the plans to improve national heath, and the imminent world shortage of animal proteins as a result of the second World War, have focused the attention of nutritionists on the high nutritive value of these products of the edible oil industry. As shown in Table 120 (see page 618), the wheat flour proteins are particularly deficient in lysine, one of the "essential" amino acids, whereas the proteins of the oil seeds are rich in this and other essential amino acids. Feeding experiments have amply demonstrated the superior biological value of the proteins of the oil seeds and their utility in supplementing the wheat flour proteins.[145-149]

---

[144] The author is indebted to J. W. Hayward, Archer Daniels Midland Co., Minneapolis, Minn., for valuable suggestions in the preparation of this section.

[145] J. A. Le Clerc, and Emily Grewe, *Soybean Digest*, **3** (1), 8 (Nov., 1942).

[146] A. A. Horvath, *Am. J. Digestive Diseases Nutrition*, **5**, 177 (1938).

[147] J. W. Hayward, H. Steenbock, and G. J. Bohstedt, *Nutrition*, **11**, 219 (1936); **12**, 275 (1936).

[148] D. B. Jones and J. P. Divine, *Soybean Digest*, 2 (11), 10 (Sept., 1942).

[149] J. W. Hayward, "The Proteins of Soybeans and Soybean Oil Meal," published in *The Composiion and Nutritive Properties of Soybeans and Soybean Oil Meal.* 2nd ed. A literature review, Soybean Nutritional Council, Chicago, 1939.

While the nutritive value of raw soybeans is low, Hayward, Steenbock, and Bohstedt[147] showed that high biological efficiency resulted from appropriate heat treatment, which caused the methionine–cystine fraction to become available. Aside from the high biological value of the proteins, the flours from these sources are rich in vitamins and minerals. Nutritionists have suggested the addition of cottonseed, peanut, and soybean flours to white flour in order to improve the nutritive value of white bread.[146–148, 150] For the past few years the production of soybean flours has averaged from 25,000,000 to 30,000,000 lbs. annually but it is estimated that the volume of various edible soy products for 1943 will approach 400,000,000 lbs; the present production of cottonseed and peanut flours is relatively small.

2. Chemical Composition

The proximate composition of cottonseed, peanut, and soybean flours, together with the available data on their content of vitamins and certain minerals, is given in Table 146; the composition of these products naturally varies somewhat, depending upon the variety, the environmental conditions under which the seeds are grown, and the extent to which they are defatted. As compared with flours milled from the cereal grains, all are much higher in protein, ash, and fiber content and lower in nitrogen-free extract.

In contrast to the cereals, the work of Osborne and of Jones and their associates has shown that the principal proteins of these oil seeds are globulins. In their studies of the cottonseed proteins, Jones and Csonka[151] obtained two globulins (α-globulin and β-globulin), a pentose-containing protein, and a small quantity of what appeared to be a glutelin. Johns and Jones[152] isolated two globulins from the peanut which they named arachin and conarachin. These proteins account for 85% of the total nitrogen of the meal and are present in the ratio of about 3 to 1, respectively.[153] Osborne and Campbell[154] showed that a salt-soluble protein, which they named glycinin, was the principal protein of the soybean. The amino acid composition of these various proteins is summarized in Table 120; in general, they are considerably higher in their content of essential amino acids than the cereal proteins and, since the total amount of protein present is also much higher, cottonseed, peanut, and soybean flours are much richer sources of the amino acids which are indispensable components of the diet.[155]

The carbohydrate content of these flours is low as compared with those from the cereals. Moreover, starch makes up a very much smaller proportion of the total carbohydrates. In fact, soybeans contain very little if any starch.[156] The carbo-

---

[150] L. H. Bailey, R. G. Capen, and J. A. Le Clerc, *Cereal Chem.*, **12**, 441 (1935).

[151] D. B. Jones and F. A. Csonka, *J. Biol. Chem.*, **64**, 673 (1925).

[152] C. O. Johns and D. B. Jones, *J. Biol. Chem.*, **28**, 77 (1916).

[153] D. B. Jones and M. J. Horn, *J. Agr. Research*, **40**, 673 (1930).

[154] T. B. Osborne and G. F. Campbell, *J. Am. Chem. Soc.*, **20**, 419 (1898).

[155] F. H. Hafner, *Bakers Tech. Digest*, **16**, No. 10, 247 (Oct., 1942).

[156] M. M. MacMasters, S. Woodruff, and H. Klaas, *Ind. Eng. Chem., Anal. Ed.*, **13**, 471 (1941).

hydrates of decorticated soybeans comprise about 12% of polysaccharides (dextrins, galactans, and pentosans) and about 12.5% of sugars (6% sucrose, 5% stachyose, and 1.5% raffinose). In the instance of the peanut, the proportions of starch and soluble carbohydrates are approximately equal.

TABLE 146

AVERAGE PROXIMATE COMPOSITION, VITAMIN, AND MINERAL CONTENT OF COTTONSEED, PEANUT, AND SOYBEAN FLOURS

Product	Moisture, %	Protein, %	Fat, %	Fiber, %	N-free extract, %	Ash, %	Fuel value per 100 g., Cal.
Cottonseed flour[a]	6.3	57.5	6.5	2.1	21.4	6.2	. . .
Peanut flour[b]	4.4	59.0	9.7	2.5	20.6	3.8	. . .
Soybean flour:[c]							
Full-fat	5.5	40.5	22.0	2.5	24.5	5.0	464
Medium-fat	5.0	50.0	7.0	2.5	30.0	5.5	380
Low-fat	7.0	53.0	0.8	2.0	31.2	6.0	344

VITAMIN AND SELECTED MINERAL CONTENT

	Cottonseed flour[a]	Peanut flour[b]	Soybean flours[d]		
			Full-fat	Medium-fat	Low-fat
Vitamins:					
Vitamin A, I. U. per g.	....	.....	1.40	1.15	0.75
Thiamine, γ per g.	10.4	6.0	6.0	7.0	7.5
Riboflavin, γ per g.	10.2	3.0	3.2	3.8	4.0
Niacin, γ per g.	85.0	189	48	57	60
Pantothenic acid, γ per g.	25.5	.....	11	13	14
Biotin, γ per g.	....	.....	0.6	0.7	0.7
Inositol, γ per g.	....	.....	1750	2050	2200
Choline, γ per g.	....	.....	2250	2250	2250
Minerals:					
Calcium, %	0.2	0.065	0.28	0.33	0.33
Magnesium, %	0.65	0.365	0.22	0.22	0.22
Phosphorus, %	1.30	0.565	0.60	0.62	0.62
Iron, %	0.012	0.010	0.02	0.02	0.02

[a] Data furnished by Traders Oil Mill Co., Fort Worth, Tex.
[b] Data furnished by National Peanut Council, Inc., Atlanta, Ga.
[c] Data from P. E. Sprague, "Edible Soy Flour," Soy Flour Association, Chicago, 1940.
[d] Data furnished by J. W. Hayward, Archer Daniels Midland Co., Minneapolis, Minn.

Cottonseed, peanut, and soybean oils (also corn oil) are somewhat similar chemically. The lipids of soybeans include from about 2 to 4% of phosphatides, expressed as lecithin. Soybeans and crude soybean oil contain substances which inhibit oxidative rancidity, but their chemical nature is at present unknown.

The heat processing employed in the manufacture of edible products from the cottonseed, peanut, and soybean largely inactivates the enzymes and greatly reduces any tendency of the oils to become rancid upon storage. In addition to other enzymes, raw soybeans contain relatively large amounts of urease, the enzyme which liberates ammonia from urea; uricase, which hydrolyzes uric acid with the formation of allantoin, and an oxidase enzyme which will oxidize the yellow pigments of wheat flour to colorless compounds. Before the development of heat-processed, edible flours, a soybean flour made by grinding whole soybeans was sold as a bleaching agent for incorporation in bread doughs; such a preparation was sold under the trade name of Wytase.

For a more complete treatment of the chemistry and technology of the soybean and its derived products, the reader is referred to the recent publication of Markley and Goss.[156a]

The vitamin and mineral analyses recorded in Table 146 show that cottonseed, peanut, and soy flours are rich sources of nutritionally important B vitamins and of calcium, phosphorus, and iron; as in the instance of cereal flours, the ratio of calcium to phosphorus is undesirably low from the nutritional viewpoint. Cottonseed flour is the highest of the three types in thiamine and riboflavin whereas the soybean flours are highest in calcium and iron content. Payne[157] has reported that peanut flour (7.0% fat) made from white Spanish-type peanuts contains 12.0 γ thiamine, 5.0 γ riboflavin, and 350 γ nicotinic acid per gram; these values are much higher than those recorded in Table 146. Booher[158] has recently published data which show that Spanish-type peanuts are higher in thiamine than the Virginia type. Doubtless, the thiamine values of these flours are also subject to variation as a result of different conditions of heat treatment.

3. Uses

Cottonseed and peanut flours are not yet produced in appreciable quantities, but it would appear that they may find similar applications in food manufacture to those which have been developed for edible soy products.[159, 160]

The processed bean meats are used as edible beans (baked or boiled) and in stews, but, in general, the soy products are blended with other foods. Aside from their nutritive value, the desirability of employing soybean flours in commercial food preparations depends on specific properties, such as their high water-absorbing and and water-retaining capacity, the emulsifying action of the lecithin, and the low carbohydrate content. High-fat flours are employed principally in bakery goods where the high fat content and consequent shortening effects are desirable. Medium-fat flours find their most extensive use in the meat-packing industry as a binder in the manufacture of sausages and other prepared ground meat products; their purpose is to prevent crumbling and to increase moisture retention. Low-fat flours are designed for use in bakery goods (bread, rolls, biscuits, etc.), prepared pancake and doughnut flours, breakfast cereals, macaroni products, dietary foods (prepared baby foods, flour and bread for diabetics), and dog foods. Large quantities of soy flours are being employed in dry soup preparations; soy flour is also an ingredient of the "K biscuit" developed by the United States Army.

[156a] K. S. Markley and W. H. Goss, *The Chemistry and Technology of the Soybean and Its Derived Products*, Parts I and II. U. S. Regional Soybean Industrial Products Laboratory, Urbana, Ill. (transferred to Northern Regional Research Laboratory, Peoria, Ill., on July 1, 1942). Mimeographed, Part I, 112 pages; Part II, 69 pages (1942).

[157] D. S. Payne, *Chem. Eng. News*, **20**, 1173 (Sept. 25, 1942).

[158] L. E. Booher, National Peanut Council, Pensacola, Florida, *Proc. 1st Annual Meeting*, **1942**, p. 25.

[159] P. E. Sprague, *Edible Soy Flour*. Soy Flour Assoc., Chicago, 1940.

[160] A. A. Levinson, *Soybean Digest*, **3** (1), 4 (Nov., 1942).

Specialty breads containing up to 20 to 30% of soybean flour have been made for some time but, as is true of bread made from admixtures of wheat flour with that of other cereals except rye flour, they have never enjoyed a wide sale. It must be emphasized that such percentages result in the production of bread different in character from that made of white flour; the bread is lower in volume, is usually darker in crust color, and possesses a darker-colored crumb of different texture and flavor. Bread containing soy flours has been found to stale less rapidly than that made entirely from wheat flour. The effect on characteristics of the bread depends on the amount of soy flour added and on the strength of the wheat flour with which it is mixed. The principal protein (glycinin) of soy flour, unlike the wheat flour proteins, does not form gluten. When used in amounts over about 5%, the doughs become more difficult to handle and their fermentation behavior differs from that of wheat flour doughs. Soy flour contains relatively large quantities of yeast nutrients (such as fermentable sugars, assimilable forms of nitrogen, and mineral salts) as well as yeast nutrilites (such as various members of the B complex); for these reasons the presence of soy flour in a yeast dough tends to stimulate gas production during the early stages of fermentation. However, heat-processed soy flours are low in amylase activity so that the rate of maltose production from starch and dextrins is less than normal; for this reason there is likely to be a slow rate of gas production during the later stages of fermentation, particularly during the proof period. These effects have recently been discussed by Hafner[161] as well as the contributions made by soy flour to the nutritive value of the bread.[155] Reference is made to the fact that the present flour enrichment program does not provide for restoration of the nutritive quality of the proteins of wheat flour to that possessed by whole wheat and that soybean flours are more economical and compare favorably with skim milk solids as a means of supplementing the wheat flour proteins as well as contributing to riboflavin and calcium content.

Millers and bakers do not look with particular favor on the use of admixtures of other flours with refined wheat flour because of their effects upon baking characteristics and on the quality of the finished bread, as judged by appearance, palatability, etc. In order that bread may meet with ready consumer acceptance, the baking industry favors the use of skim milk as a means of supplementing the wheat flour proteins and of increasing the riboflavin and calcium content; moreover, the incorporation of milk solids in a dough does not require any considerable adjustments in baking procedure. An increase in the nutritional knowledge of the consumer may result in greater emphasis being placed on nutritional aspects of foods by the buying public in the future.

[161] F. H. Hafner, *Bakers Tech. Digest*, **16** (12), 282 (Dec., 1942).

Selected Bibliography

Bailey, C. H., *The Chemistry of Wheat Flour.* Chemical Catalog Co., New York, 1925.

Bailey, C. H., *The Constituents of Wheat and Wheat Products.* American Chemical Society Monograph No. 96, Reinhold, New York, 1944.

Barger, G., *Ergot and Ergotism.* Gurney and Jackson, London, 1931.

Gortner, R. A., *Outlines of Biochemistry.* 2nd ed., Wiley, New York, 1938.

Hopkins, R. H., and Krause, C. B., *Biochemistry Applied to Malting and Brewing.* Van Nostrand, New York, 1937.

Hutcheson, T. B., Wolfe, T. K., and Kipps, M. S., *The Production of Field Crops.* McGraw-Hill, New York, 1936.

Kent-Jones, D. W., *Modern Cereal Chemistry.* 3rd ed., Northern Pub. Co., Liverpool, 1939.

Morrison, F. B., *Feeds and Feeding.* 20th ed., Morrison Pub. Co., Ithaca, 1940.

Osborne, T. B., *The Vegetable Proteins.* Longmans, Green, New York, 1912.

Schulerud, A., *Das Roggenmehl.* Moritz Schäfer, Leipzig, 1939.

Sherman, H. C., and Pearson, C. S., *Modern Bread from the Viewpoint of Nutrition.* Macmillan, New York, 1942.

Winton, A. L., and Winton, K., *The Structure and Composition of Foods.* Vol. I, Wiley, New York, 1932.

Chapter XVIII

BAKING AND BAKERY PRODUCTS

WILLIAM H. CATHCART

New York, N. Y.

Since the making of the first bakery products, baking has been considered an art. Although today, to a great extent, it is still an art, science and engineering have made possible the development of large, mechanized, sanitary plants where baked goods of uniform quality are produced day after day. Morison[1] has reviewed the early work of the application of science to baking.

Although the first scientific work as applied to baking took place 175 years ago, it has been only recently that the baking industry has shown an interest in what chemists and other scientists can do for it. It must be admitted that the baking industry was the last branch of the fermentation industries to institute a systematic, scientific study of materials used and processes taking place during manufacture. Today, however, many of the large bakeries of this country have their own laboratories; and there are commercial laboratories specializing in bakery problems whose services are available to smaller bakers. In fact, the baking industry as a group has shown its interest in scientific development by its support of laboratories and a technical school operating under the name of "The American Institute of Baking."

I. PROGRESS OF THE BAKING INDUSTRY

Baking is fast becoming a scientific procedure in the true sense of the word, because all processes of baking involve complex physical, chemical, and biological changes, which can be segregated and explained only by the scientist. Already, science has done a great deal to bring many of these complex factors under control.

So far, science has not revolutionized the baking industry; it has just made the baker's job simpler. The increased ease with which the baker produces uniform products meeting high standards has been brought about mainly by the development of new and improved ingredients and the use of machines that utilize modern principles of engineering.

1. Ingredients

Flour has been standardized and improved, and methods for its evaluation have been worked out. Special machines employing principles of modern physical and colloidal chemistry have been developed for measuring the elasticity and strength of protein in flour, the gas-producing power of flour, and the gas-retaining power of flour. Diastatic and proteolytic activities of flour are being controlled.

---

[1] C. B. Morison, *Ind. Eng. Chem.*, **20**, 1292 (1928).

Milk has been standardized and processed so that it gives uniformly good results when used in bread and other bakery products. Spray-dried, roller-dried, and sweetened condensed skim milks are the most common types used.

Standardized preparations of diastatic malt, mineral yeast foods, and dough conditioners are available. The malt preparations contain diastatic and proteolytic enzymes with which the flour or doughs can be fortified if necessary. The yeast foods and dough conditioners contain ammonium, calcium, and/or phosphate compounds to help feed the yeast and overcome soft water conditions in certain localities. These latter preparations generally contain a very small quantity of an oxidizing agent, such as bromate, which helps to stabilize the dough and make it better able to retain gas.

Yeast itself has been made uniform. This alone is a most important factor in the progress of the industry. Uniform yeast-raised products are not possible unless uniform yeast of definite strength is available.

Sugar and salt do not need any special preliminary treatment for the baker.

Special shortenings have made their appearance, developed for the particular bakery products for which they are intended. The baker uses today what were formerly useless vegetable oils. These oils have been converted into plastic fats, some for use in bread, others with high emulsifying power for use in cake. Fats of high stability have been made availabe for baking crackers, and frying doughnuts, potato chips, and popcorn. Certain fats, like lard and regular hydrogenated cottonseed oil, have been segregated for their high shortening power for use in flaky pastry such as pie doughs. All of these products are available, and are alike from shipment to shipment.

High-grade frozen eggs have been a great step forward for the baker. Such eggs are more uniform in quality than fresh eggs.

Scientists have developed special baking powders for the commercial baker. The newer baking powders are termed "slow acting." Their importance lies in the fact that they release little carbon dioxide gas until the product reaches the oven. The acid component of such baking powders is a mixture of several acid salts; one such baking powder contains pyrophosphates which do not react to a significant degree until heated. Cakes containing these baking powders can be handled through machines without losing too much carbon dioxide from the baking powder. The development of these powders, together with refrigeration, has made it possible for the baker to give his customers fresher products, for he can bake them as he needs them.

The scientist has also played an important role in the development of new products and new processes, in that he has made possible the addition of vitamins and minerals to breads, as well as the production of sanitary, economical, and practical packages in which to distribute the baked goods. Science has also pointed the way for the control of mold and bacterial infections which spoil bakery products.

2. Laboratory Control

The methods which baking laboratories use for control purposes are to be found in publications of the Association of Official Agricultural Chemists[2] and the American Association of Cereal Chemists.[3] Thus, recognized methods are employed. However, special practical tests must be used at times, as is the case in any industry.

Many of the ingredients that go into bakery products change from season to season. This is particularly true of flour, and the baker must be extremely alert to the adjustment and handling of all of these materials from crop to crop so that uniform products can be obtained. This means that continuous quality control tests must be exercised on ingredients and products at all times. Thus, laboratory control in connection with bakery products is of vital importance.

Bread products have been standardized to the point that federal definitions have been set down for most of them, as, for example, white bread, enriched bread, milk bread, raisin bread, whole wheat bread, and wheat and white bread.[4]

II. PROCESSES FOR YEAST-RAISED BAKERY PRODUCTS

Yeast-raised bakery products are, in general, made by two processes: the sponge and dough method, and the straight dough method. The sponge and dough method is used in the main for the production of bread, rolls, and crackers. The straight dough method is generally used for the production of yeast-raised bakery products, such as sweet rolls and coffee cakes. Either process, however, can be used for producing all these products. The small retail baker generally uses the straight dough method only, since it requires less handling.

In the sponge and dough method some of the ingredients—the flour (50 to 70% of the total flour is added at this point), water, salt, yeast, dough conditioners, and malt (if any)—are mixed and fermented. After the proper length of fermentation, which is approximately $4^1/_2$ hrs. for bread and 24 hrs. for crackers, the sponge is put back into the mixer and the rest of the ingredients are added, along with some of the flour that was held out from the sponge stage, and remixed. After a very short fermentation, the dough, which is formed by the second mixing, is ready for processing.

In the straight dough method, all the ingredients are mixed at once, then fermented, during which time the gases are expelled at intervals by cutting the dough over. At the end of the fermentation period, the dough is processed without remixing.

Comparison of Formulas for Yeast-Raised Products

In practice, the only difference between various yeast-raised products is their richness, that is, the amount of sugar, shortening, milk, and eggs used. A comparison of the various ingredients is given in Table 147.

[2] Assoc. Official Agr. Chem., *Methods*, 5th ed., Washington, D. C., 1940.

[3] Am. Assoc. Cereal Chem., *Cereal Laboratory Methods*, Lincoln, Neb., 1935

[4] *Federal Register*, **8**, 10780 (1943).

The leanest products are crackers and whole wheat bread. Average white bread contains more sugar and shortening than crackers or whole wheat bread, and in addition contains some milk. Rolls contain more shortening, sugar, and milk than white bread. Sweet yeast-raised products contain still more sugar, shortening, and milk and in addition have eggs added. It should be noted that as the richness of the formula increases, the quantity of the yeast is also raised in order to keep fermentation going at a fast rate.

TABLE 147

COMPARISON OF FORMULAS FOR YEAST-RAISED PRODUCTS

Figures Are Percentages on Basis of Flour as 100%—Only Basic Ingredients Shown

Ingredient	Crackers	Whole wheat bread	White bread	White rolls (dinner and hamburger rolls, etc.)	Sweet yeast-raised products (sweet rolls, coffee cakes, etc.
Flour (diastated)	100	100	100	100	100
Water	56	66	62	64	60
Yeast	1.5	2	2	3	4
Salt	1.75	2.25	2.25	2.25	2
Sugar	1[a]	4	6	8	14
Milk (dry skim)	0	0	4	6	6
Shortening	5	5	4	8	12
Eggs (whole)	0	0	0	0	10
Dough conditioners	0.1–0.3	0.2–0.4	0.2–0.5	0.2–0.5	0–0.25

[a] Optional.

Thus, the following discussion on fermentation applies to all of these bakery products.

III. GAS PRODUCTION IN YEAST-RAISED DOUGHS

Gas production brought about by yeast growth in dough is necessary for the production of light porous bread, rolls, coffee cake, and crackers. Yeast, being a living plant, requires food and moisture before it will grow and produce gas. Since a dough necessarily contains sufficient moisture for the growth of yeast, additional factors are sugars and other substances that either directly or indirectly supply the yeast with food. These latter factors include ammonium (nitrogen) compounds, calcium and phosphorus compounds, diastatic enzymes, dextrins, and soluble starches.

1. Yeast

Yeast itself is of prime importance in gas production, and the amount of gas produced is directly proportional, at least in the first few hours of fermentation, to the quantity of yeast added to the dough. Sufficient amounts of yeast must be added initially to supply gas during the total fermentation time, provided sufficient food is available in the dough.

The type or species of yeast is also important. Different types of yeast produce definite volumes of gas at different rates; thus, some will produce more gas in a given length of time. However, most of the compressed yeasts offered to the baking industry at the present time are similar in their action.

When considering yeast as a prime factor in gas production, it is important that the time and temperature at which the yeast is allowed to work be taken into consideration. The longer the time of fermentation and the greater the temperature of fermentation, the greater the quantity of gas produced.

Yeast produces gas by the production of the enzyme complex, zymase. Zymase is necessary for the conversion of so-called "simple sugars" such as dextrose (corn sugar) and fructose (fruit sugar) into alcohol and carbon dioxide gas. The more zymase produced by the growing yeast, therefore, the more gas is produced in the dough, provided an ample amount of simple sugars is present.

2. Sugars

Second in importance in the production of gas in doughs are sugars. Sugars are necessary, as pointed out above, to furnish material which zymase of the yeast can turn into alcohol and carbon dioxide gas. The amount of sugar present is, of course, of importance. If an insufficient supply is present in the beginning, gas production will continue until all of the sugar is exhausted and gas production will then slow down and in some cases almost stop.[5]

There is 1 to 2% of sugar originally present in flour. Thus, dough, without any added sugars, starts to ferment at about the same rate as a dough with added sugar. But the nonsugar dough will soon begin to slow down, and its rate of gas production will be much less than one with added sugar, unless considerable diastase (discussed below) is present.

There are many kinds of sugars that can be used in doughs, one of the most common being sucrose. This sugar cannot be utilized directly by yeast zymase, but must first be inverted by another enzyme found in the yeast, sucrase (or invertase):

$$\underset{\text{(cane or beet sugar)}}{\text{Sucrose}} + \text{water} + \underset{\text{(in yeast)}}{\text{sucrase}} \longrightarrow \underset{\text{(simple sugars)}}{\text{dextrose} + \text{fructose}}$$

Once sucrose is broken down into the above simple sugars, the enzyme zymase can produce alcohol and carbon dioxide gas.

Another type of sugar that can be utilized very readily is malt sugar, which is found in malt extract. This sugar is known as maltose and again cannot be utilized directly by zymase. It must first be broken down into the simple sugar, dextrose, before zymase can use it to produce gas. Dextrose is produced from maltose by the enzyme maltase, also found in yeast.

---

[5] Too much sugar will also slow down gas production. This is very evident when more than 10% of sugar is used in a dough, and is the reason why a greater percentage of yeast must be used in sweet yeast-raised goods than in bread.

Honey and invert sugar can be utilized directly by yeast zymase because they are simple mixtures of the simple sugars, dextrose and fructose.

Lactose, found in milk, commonly called milk sugar, is not utilizable for the production of gas. Since there is no substance in the dough that will break down lactose to simple sugar, yeast zymase cannot use it for the production of gas. It remains as such in the dough but does help (by caramelization) to give color to the finished product.

Corn sugar, or dextrose, can be used directly in the dough for the production of gas. As mentioned above, dextrose is a simple sugar and can be acted upon directly by the enzyme zymase. From this it might seem that a dough made with dextrose, or other simple sugars, would ferment much more rapidly than one made with sucrose, which must be changed to simple sugar before it can be used for gas production. However, after the first minute or two of fermentation, doughs made with the two sugars ferment at the same rate because the conversion of cane sugar to simple sugar is very rapid.

3. Ammonium (Nitrogen), Calcium, and Phosphorus Compounds

In order to be able to produce the zymase, yeast must be in an active, "growing state;" thus, ammonium (nitrogen), calcium, and phosphorus compounds must be available, for yeast needs these substances in order to grow. In general, there is sufficient calcium and phosphorus in a dough to give active yeast growth and, therefore, sufficient zymase. Accordingly, little increase in gas production is noted when additional calcium and phosphorus compounds are added to a dough. These two compounds, nevertheless, are nearly always component parts of so-called bread improvers or dough conditioners, such as Arkady.

Because there are not sufficient soluble ammonium compounds in a dough, a slight addition of ammonium compounds shows rapid increase in gas production. Most bread improvers and dough conditioners contain a small quantity of ammonium compounds which cause these preparations, when added to a dough, to increase the rate of fermentation.

4. Diastatic Enzymes

Diastatic enzymes are an indirect factor in the amount of sugar which is going to be available in a dough for yeast consumption. Diastatic enzymes are found naturally in flour, in malted wheat, malted barley, and malt extract itself. They are not found in yeast.

Diastase acts on starch and changes starch to maltose sugar.[6] Maltose sugar in turn, as mentioned above, can be changed into simple sugar or dextrose by the enzyme maltase found in yeast. These changes are outlined as follows:

---

[6] Diastase will not act on the starch granule until another enzyme, cytase, removes the cellulose covering the granule. Since there is apparently sufficient cytase available in a dough, the removal of the cellulose coating does not limit the action of the diastase.

$$\underset{\text{(in flour)}}{\text{Starch}} + \text{water} + \underset{\text{(in flour and malt)}}{\text{diastase}} \longrightarrow \underset{\text{(malt sugar)}}{\text{maltose}}$$

$$\underset{\text{(malt sugar)}}{\text{Maltose}} + \text{water} + \underset{\text{(in yeast)}}{\text{maltase}} \longrightarrow \underset{\text{(corn sugar)}}{\text{dextrose}}\ \text{(simple sugar)}$$

$$\text{Simple sugars} + \text{water} + \text{zymase} \longrightarrow \text{alcohol} + \text{carbon dioxide gas}$$

It has been general practice in the baking industry for some time to control the rate of gas production of a dough by the addition of malt extract containing diastase.

The temperature at which the dough is fermented affects the rate at which enzymes such as diastase will catalyze the conversion of starch into sugar. The higher the temperature, the greater the rate of action. When the temperature reaches approximately 150° F., however, the activity of the diastase is inhibited and the chemical compounds which constitute diastase will decompose.

The ideal situation in fermentation is to have diastase producing sugar from the starch at about the same rate that the yeast is using the sugar. In this way, most of the sugar added initially will be present at the end of fermentation to give sweetness, desirable crust characteristics, etc., to the bread.

The production of carbon dioxide gas from starch, as has been shown, is entirely enzymatic. The enzymes that carry through these characteristic reactions work best at a *p*H of approximately 4.7. A bread dough, after about a 3-hr. fermentation, has approximately the proper *p*H for optimum fermentation.

During the conversion of starch into maltose, several products are produced before maltose, the most important being dextrins and soluble starch.

$$\underset{\text{(in flour)}}{\text{Starch}} + \text{water} + \underset{\text{(in flour and malt)}}{\text{diastase}} \longrightarrow \text{soluble starch} \longrightarrow$$

$$\text{dextrins} \longrightarrow \underset{\text{(malt sugar)}}{\text{maltose}}$$

5. Dextrins and Soluble Starch

There are many preparations offered to the baker containing dextrins and soluble starch, which are supposed to improve bread. In a dough containing sufficient diastase, sugar can be produced from the original starch almost as readily as from the dextrins or soluble starch. However, if insufficient diastase is present, dextrins and soluble starch will increase gas production. This same effect is accomplished by overgrinding flour. Overgrinding (too fine grinding) ruptures some of the starch granules and makes the starch more available to diastatic action.

As the quantity of dextrins produced by diastase in a dough increases, the moistness of the crumb of the finished bread also increases. If the amount of diastase becomes too great, there is too much dextrin produced and the loaf will have a sticky and damp crumb.

Kent-Jones and Amos[7] describe a test to determine the amount of dextrin in a loaf of bread.

D. W. Kent-Jones and A. J. Amos, *Cereal Chem.*, **17**, 265 (1940).

6. Effect of Milk on Diastase

Milk, when added to a dough, retards diastatic action because it increases the *p*H of a dough, shifting the *p*H away from the optimum point of activity. This retarding of diastatic activity by milk is reflected in the gas production, that is, a decrease in gas production of doughs containing milk. This can be corrected by the addition of more malt extract, vinegar, or calcium acid phosphate, and can be helped by the addition of sugar.[8] If the *p*H is adjusted by adding a small quantity of acid or acid salt, a dough containing milk will actually show an increase in gas production because milk increases zymase activity.

7. Control of Diastase in Flour

Diastase can be added to a dough through the flour, as well as by malt extract. It is a general policy now for millers to add malted barley and/or malted wheat flour to the regular flour in order to increase its diastatic activity to a certain level. If this is increased far enough, no malt extract need be used by the baker.

The diastatic activity of flour can be measured by gas-production tests or by the so-called "maltose number," the number of milligrams of maltose produced in one hour at 30° C. by 10 grams of flour in suspension. The gas-production test consists of making small doughs containing no sugar, fermenting them, and measuring the amount of gas given off.[9] This gives a test that is comparable to the gas production which would be obtained in a commercial dough.

The general practice of controlling the amount of diastase in flours has the advantage that the baker needs to handle one less ingredient and can be sure that the gas production of his doughs will be adequate. Also, there is no danger of getting too much diastase in the dough.

8. Gas-Production Tolerance

As mentioned in the above discussion, a dough must be able both to manufacture enough gas to produce a well-aerated loaf of bread and to maintain a high rate of gas production over a considerable period of time. In fact, gas production must be at its maximum at the time the dough is ready to go into the oven. It must be sufficient so that, if it is necessary to allow the dough to ferment longer than usual, the gas-production rate will not fall off to any great extent, but will remain at or near the optimum level.

The easiest way to obtain such tolerance in a dough is by the use of sufficient diastase.

Gas production is of little value in a dough unless the dough has the properties to retain the gas produced. Thus, gas retention goes hand in hand with gas production; it is just as important and should receive the same consideration.

---

[8] O. Skovholt and C. H. Bailey, *Cereal Chem.*, **14**, 108 (1937).

[9] R. M. Sandstedt and M. J. Blish, *Cereal Chem.*, **11**, 368 (1934).

IV. GAS RETENTION IN YEAST-RAISED DOUGHS

Proteins in wheat flour are responsible for retaining gas in all yeast-raised products. These proteins, when mixed with water, have the property of forming a rubbery mixture known as gluten. Wheat is the only cereal which contains proteins (gliadin and glutenin) having the property of forming this glutinous mass. Thus, other cereals are not used for preparing baking flours. When other cereals such as rye flour are used in yeast-raised products, they are simply so much dead weight added to the mixture. In such mixtures, sufficient wheat flour must be present to form enough gluten to retain the gas during fermentation. Without wheat flour, therefore, we would not have leavened bread.

The ability of a dough to retain gas is directly related to the amount and quality of wheat protein in the dough and factors which affect the elasticity of this protein. The main factors are the amount of mechanical action, the content of protease enzymes, the degree of oxidation (bleaching and maturing), and the quantity of milk.

1. Gluten Proteins

The amount of protein in the dough depends upon the protein content of the various materials used, especially of the flour and milk.

It is realized that one can have a large quantity of flour proteins without sufficient elasticity to retain gas. In general, however, the gas-retaining ability of doughs made from high-quality wheat flour is directly proportional to the amount of gluten protein. When milk is added to doughs, the protein of the milk tends to combine physically with the flour proteins and thus strengthen the flour.[10] The combined quality and quantity of the gluten proteins of the flour are of prime importance in determining the gas-retention properties of doughs made from the flour.

2. Mechanical Action

The ability of flour proteins to hold gas depends to some extent on the mechanical agitation given the dough during the mixing period and subsequent operations. Some mechanical action is necessary in order to combine physically the flour proteins into gluten. It is necessary that gluten be formed before any gas is retained. *In order to produce an elastic gluten that will hold gas, the dough is mixed for a longer time than is necessary merely to incorporate all ingredients.* The time is determined in the bakery by trial and error, final judgment being made on the finished products. In the laboratory, this can be determined quite accurately by a recording dough mixer, such as the farinograph (Fig. 59). This machine shows the amount of mixing necessary to develop the dough.[11]

[10] L. J. Bohn and C. H. Bailey, *Cereal Chem.*, **14**, 335 (1937).

[11] C. H. Bailey, *Wheat Studies Food Research Inst.*, **16**, 243 (1940).

3. Protease Enzymes

Proteases are found in flour, malt extract, malted wheat flour, and malted barley flour.

The quality or elasticity of the gluten proteins is influenced directly by the amount of proteases in the dough. They tend to decompose gluten proteins into simpler substances such as peptones, peptides, and amino acids, and thus condition the gluten. These decomposition products do not have gas-retention properties. Now, if too great a quantity of proteases is present in the dough, too great a quantity of gluten proteins will be broken down, and thus the dough will not be able to retain gas. The result amounts to a "sticky dough." On the other hand, if insufficient quantities of proteases are present, the gluten proteins are tough and nonelastic, and the baker calls the doughs "bucky." Thus, the aim is a protease content in the dough maintained in a balance which gives it elasticity and yet does not break it down and make it sticky.

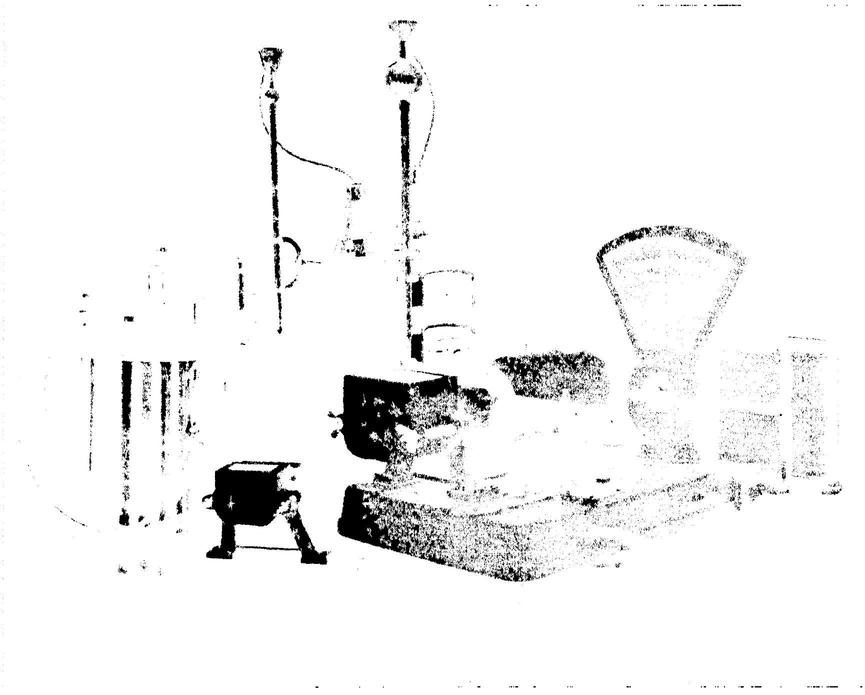


Fig 59 —The recording dough mixer or farinograph The recording of the dough being mixed is shown on the right The constant temperature water bath on the left supplies constant temperature water for the mixing bowl (Courtesy of Brabender Corporation)

When the miller adds malted wheat flour to flour to increase the diastatic activity and/or when the baker adds malt extract to the dough for the same purpose, considerable quantities of proteases necessarily are introduced into the dough at the same time. Thus, too much malted wheat flour and/or malt extract cannot be used because of the danger of introducing too great an amount of protease enzymes.

In general, doughs have too high a protease content instead of not enough. "Green flour" is an example of the former condition, and results in sticky doughs.

4. Oxidation (Bleaching and Maturing)

The amount of protease can be controlled indirectly by means of oxidation, that is, by addition to the flour and to the dough of chemical substances known as oxidizing agents. Such substances are the materials the miller uses for bleaching and maturing flours, and potassium bromate found in dough conditioners. These oxidizing agents, in some manner or other (not clearly understood at the present time), tend to inhibit the activity of proteases.[12–16] Thus, by adding oxidizing agents to flour, and/or directly to the dough, the protease activity can be reduced, and in this way can be adjusted so that normal elastic doughs are obtained.

Too much oxidation, that is, too much bleaching by the miller, or too much bromate, will produce bucky doughs, that is, doughs with insufficient protease activity. Insufficient oxidation generally means too great a protease activity and results in sticky doughs that likewise do not retain sufficient gas.

Green flours, which have too much protease activity, need more oxidation. Therefore, such flours, when used to make doughs, can stand more bromate than normal flours.

The protease activity of bread flours is thus being controlled, to a great extent, by the miller's addition of bleaching and maturing agents. Optimum protease activity of the resulting doughs is maintained. This is generally so adjusted that the additional oxidation from the small amount of bromate that the baker uses is beneficial and not detrimental.

5. Effect of Milk

Milk which has not been prepared especially for baking generally has a detrimental effect on baking products. This is due to the fact that milk contains substances which increase the activity of proteases, the resulting doughs having too much protease activity and therefore not holding the gas as well as doughs without milk.[17, 18]

---

[12] There has been considerable discussion by research workers as to whether this is the true effect. The action may be on the pentosans, the gluten, or the so-called activators of the proteases. It is possible that a combination of effects exists which accounts for conflicting data.

[13] J. C. Baker, H. K. Parker, and M. D. Mize, "The Pentosans of Wheat Flour," Presentation at Annual Meeting Am. Assoc. Cereal Chemists, Chicago, 1942.

[14] J. W. Read and L. W. Hass, *Cereal Chem.*, **16**, 60 (1939).

[15] H. Jørgenson, *Cereal Chem.*, **16**, 51 (1939); *Mühlenlab.*, **5**, 113 (1935).

[16] A. K. Balls and W. S. Hale, *Cereal Chem.*, **15**, 622 (1938).

[17] O. Skovholt, *Food Industries*, **9**, 132 (March, 1937).

[18] O. E. Stamberg and C. H. Bailey, *Cereal Chem.*, **19**, 507 (1942).

However, it was found many years ago that, if one heated the milk to near the boiling point, the substances increasing protease activity are destroyed; normal doughs, therefore, can be obtained from the use of such milk. The oldest cookbooks call for "scalded milk" in bread recipes. Just what is destroyed when milk is scalded is not clearly understood even today. The problem needs considerable investigation. Reliable producers of bakers' milk are heat treating their milk (approximately 180° F. for 30 min.), so that it will have good baking qualities.

Milk generally should not be purchased from a supplier who makes milk only for ice cream manufacturers, for instance. His milk as a rule will be of high quality, but will not have those properties necessary for the production of good bakery products.

6. Effect of Miscellaneous Factors

The elasticity of gluten and its consequent ability to hold gas is affected by the kind of water used in the dough, by the types of mineral salts added, by acids, either added or produced by fermentation, by the alcohol produced by fermentation, and by fats.

Soft water will tend to produce a sticky dough, while hard water will produce a dough with more elastic properties.

Mineral salts, such as calcium sulfate, sodium chloride, and calcium acid phosphate, tend to "stabilize" the gas-retaining properties of the flour gluten. All these mineral salts, when used in the proper quantities, give a dough the property of retaining gas over a longer period of fermentation.

Acids, either present in a dough due to fermentation or actually added, such as through the addition of vinegar, tend to break down and actually dissolve the protein. Too much acid is therefore undesirable, as it will dissolve and break down the protein to such an extent that the dough will not hold sufficient gas.

Alcohol has the same effect on gluten as acids except to a smaller extent.

Fats, that is, shortenings, to some extent make it possible for a dough to retain gas better. This is noted especially where large particles are included in the dough, and is quite obvious in the case of whole wheat doughs. Whole wheat doughs with shortening included produce loaves of much larger volume than doughs without shortening.

7. Method of Measuring Gas Retention

The oldest method of measuring gas retention, the baking test, is still in use today. The gas-retention properties of a dough are directly reflected in the volume of the resulting loaf of bread, provided sufficient gas production is maintained. In experimental test baking, doughs are made which have optimum gas production; thus the resulting volume of the loaves reflects the gas-retention properties of the dough.

Within recent years, mechanical devices have made their appearance for measuring the elasticity of doughs and the gas-retention properties. Most of these machines actually measure elasticity and only a few measure the amount of gas retained in the dough.[19] Machines of this nature have not found wide acceptance in the United States, and in general it is considered that the baking test is superior to any or all of the mechanical devices. It is to be understood, however, that some of these devices give accurate information and are definitely helpful.

8. Gas Retention Tolerance

Doughs must not only have the ability to retain sufficient gas for the production of high-quality products, but must also be able to continue to retain this quantity of gas over a considerable length of time. The combination of the tolerance of gas production with that of gas retention is known generally as fermentation tolerance. The ideal dough has good fermentation tolerance because it has good gas-production tolerance and good gas-retention tolerance.

V. SOUR RYE BREAD

There is a great variation in the quality and flavor of the various rye breads manufactured by bakers in this country. Many European nationalities are represented in the United States, all accustomed to eating different types of rye bread. Some people may prefer the light, mild ryes; some, especially the Scandinavians, like a coarse-grained, sweet-tasting loaf; and some (among them those of German or Jewish descent) prefer a fairly dark, mildly sour rye loaf.

Sour rye breads owe their delicious and distinctive taste to the fact that they are slightly sour. They differ from every other bakery product in that they contain more acid. A "tangy" or "sour" rye must have a pH value of from 4.3 to 4.7. As a comparison, white bread has a pH range from 5.2 to 5.5. In order to obtain this greater acidity, so-called "sours" are used. Sours are "ferments." They were used for centuries without any understanding of the mechanism of their activity. Their preparation was more or less a carefully guarded family secret, for the sours imparted a special flavor to the bread on which was based the reputation of the family for quality rye bread.

It is of considerable interest to follow the development of the rye sours, as they are undoubtedly the most important rye bread flavors, at least for the production of tangy-flavored rye breads.

1. Old Method of Production of Sours

The old method of producing sour rye breads involved the starting, building up, and rebuilding of a dough mass in which natural bacteria developed acids (mainly lactic and acetic) and other substances which gave the final bread a delightful tangy flavor.

First a small dough or starter consisting principally of rye flour and water was prepared and allowed to ferment for 5 to 10 hrs. This dough was then refreshed several times (each time allowing the dough to ferment 5 or 6 hrs.) with additional flour and water until sufficient acid was present, during which time the souring organisms developed the flavor. Portions of this basic sour were incorporated in the sponge or dough and the regular procedure for breadmaking followed. As no definite bacteria were added to the original starter, the souring depended entirely on the few natural bacteria in the flour and water and on a few from the air. These old-style sours, therefore, were definitely not uniform.

---

[19] C. H. Bailey, *Wheat Studies Food Research Inst.*, **16**, 243 (1940).

Part of the basic sour was always saved and rebuilt into another basic sour. This helped to shorten the time of development, but did not contribute greatly to uniformity. As stated above, the rebuilding of rye bread sours in some bakeries was continued for generations and guarded as a family secret. However, the basic sour was not necessarily the same from time to time. Changes in temperature would cause some bacteria to grow and would inhibit others, etc. Foreign bacteria and wild yeast would get in from the air and cause gradual changes and deterioration in the flavor. The baker had little control over the sour.

Some bakers added yeast to the starter, hoping thus to give a more vigorous start to the souring. The yeast helps somewhat, but is of little value in producing acid.

In present-day companies with a uniform standard of quality to maintain, the uncertain method outlined above is not suitable. During the last 20 years, in fact, many methods have been tried in order to obtain at all times uniform products.

2. General Method for Controlled Sours

Controlled cultures are made by adding a definite number of the desired type of bacterium to the dough mass to be used as the sour. If a large amount of a particular bacterium is inoculated into a dough mass, this bacterium will tend to outgrow others and thus there will be few, if any, contaminating organisms. The inoculated dough mass is then allowed to ferment under controlled conditions of temperature and humidity until the desired amount of acid is developed. The proper temperature must be maintained for the development of the specific organisms desired: In the case of acetic and lactic acid producing bacteria, which are the types desired, temperatures around 80° F. should be maintained; if the fermentation temperature is above 90° F., butyric acid producing organisms may predominate. Butyric acid and other substances produced at the higher temperatures are undesirable, and result in a bad-tasting sour. Therefore, if optimum temperatures are maintained and if the sour is inoculated with a large number of the desired organisms, a pure culture (from a practical standpoint) will be obtained.

Some of the sour is saved and renewed. The uniformity of this method depends on starting completely anew about every 5 days or sooner, and on the available source of the original bacteria. The more often the sour is started, the more uniform the results.

There are several sources for the starting bacteria. Acetic acid producing bacteria can be obtained from unpasteurized cider vinegar. Lactic acid producing bacteria can be obtained from unpasteurized buttermilk, Bulgarian buttermilk, cultured buttermilk, or acidophilus milk. One cannot be sure of the concentration of bacteria in most of these different sources; since the concentration is variable, the resulting sour will show variation. Unless the sour is restarted quite often, further variations will ensue.

Within the last few years, certain bakery suppliers have made so-called rye cultures (semidry preparations of acetic and lactic bacteria) available to the baker, enabling the baker to start his sours every day and assuring him of uniform products.

VI. CAKE AND COOKIE PRODUCTION

Much progress has been made in the past 20 years in the production of cake, particularly in the development of scientific reasons for performing certain operations and in the development of better ingredients and processes. The scientific literature actually contains very little work on the technology of cake and cookie baking, most of the scientific development in this field having come from the suppliers of the ingredients. Many of the processes and products are patented. Since they have never been written up in the literature, the writer is taking the liberty of discussing this subject briefly without reference to citations to the literature or to patents. For the most part, the information comes from his own experience and that of his coworkers.

1. General Aspects

In many respects the production of cake, doughnuts, and cookies is similar to that of bread and other yeast-raised products, that is, the problem is one of gas retention and gas production: The batter or dough must be such that it will retain gas; and the batter or dough must contain a sufficient quantity of gas-producing agents.

The latter problem is much less complicated with cakes and cookies than with bread and other yeast-raised products. It is a simple matter to use an adequate amount of the right type of leavening agents for cakes and cookies. The former problem, however, is probably more complicated in this case than for yeast-raised products. The ingredients must be so adjusted that they form a stable, colloidal, emulsion-like mixture that will retain gas as long as it is being produced.

Cake doughnut batters are similar to pound cake batters; cake doughnut batters, however, form more gluten and most of the fat comes from the frying process. Nevertheless, they should be considered with cake batters.

For cookies a dough rather than an emulsion is used; one is thus more dependent upon gluten for the retention of gas than in the case of cakes. Enough gluten should be present so that the dough has sufficient structure; but if too much is present the dough will not have sufficient spread in the oven.

2. Leavening

If a cake or cookie batter were not leavened, that is, were not to rise during baking, the resulting product would be dense, tough, and unpalatable.

The following leavening means are used for cakes and cookies: beaten eggs; baking powder; ammonia; and creaming.

Leavening by beaten eggs is used in producing such cakes as angel food and sponge. In the first, egg whites are used, and in the second, egg yolks. Both depend upon the beating of the eggs to a foamlike mass due to the incorporation of air, and so adjusting the batter that this foamlike mass will be stable until the cake has been baked and the protein or gluten "set" by the heat of the oven.

Baking powder is used in most cakes, other than angel food and sponge cakes. The type of baking powder used by the commercial baker is so adjusted that it releases little gas until it reaches the oven. Baking powder action is always coupled with creaming so that the total leavening is due to the gas released by the baking powder and also to the expansion of the air incorporated during the creaming or mixing of the cake. Creaming without baking powder is used in pound cake; however, a small amount of baking powder is added to most present-day pound cake batters.

Ammonia, that is, ammonium bicarbonate, is used for leavening certain types of cookies. During baking the ammonium bicarbonate breaks down into carbon dioxide, ammonia, and water vapor. Ammonium bicarbonate as leavening is used in many cookies because of the "spreading" properties it gives the cookies and the fact that it also contributes to the color of the cookies—that is, the chemical action in itself and the *p*H resulting during release of the gas help to give the cookie a more desirable color.

As gas-retaining properties of the dough or batter decrease, the amount of leavening must be increased. Also, as the altitude at which the cake is being made increases, the amount of leavening used should be decreased.

3. Cake and Cookie Flours

Although flour is discussed at other points in this volume, it is desirable to discuss briefly, at this point, the type of flour necessary for cake and cookie production.

Cake and cookie flours are milled from soft wheats. For producing tender cakes with a soft texture, flours milled from soft winter wheats are definitely better than those milled from hard wheats because soft wheat flours have less protein, and this protein is of a "softer" nature than that in hard wheat flours.

The shortest "separation" soft wheat flours, that is, those having the lowest quantity of protein and the highest quantity of starch, are used for foam-type cakes and high sugar content cakes. Flours used for the heavy cakes, such as pound, fruit, and loaf cakes, have a slightly higher quantity of protein and a correspondingly lower quantity of starch.

There are differences in cake flours due to factors other than quantity of protein and starch. There are differences due to the inherent quality of the type of wheat from which the flour is milled. There are differences due to the type and amount of aging (maturing) used, granulation, and protein content.

The following discussion deals with properties of cake and cookie flours that can be controlled by the mill.

(*a*) *Absorption*

Absorption, which is associated with bread flour, can be described as the liquid-carrying capacity of cake flours. High liquid-carrying capacity is important, especially on high-sugar cakes. The flour must also have the ability to retain moisture in the finished cake.

Absorption, when used in connection with cookie flours, is still liquid-carrying capacity. It is, however, somewhat more closely related to absorption of bread flours. This is true because, when making cookies, a dough instead of a batter is actually formed.

(*b*) *Protein*

In bread, the gluten protein is the framework of the loaf. The gluten is not of such importance in cake. The protein content of cake flours should be low so that tender products will be formed; high-protein cake flours produce tough eating products. There is little gluten formed in cake mixtures (due to large amounts of water in the mix and the bleaching treatment given the flour), but that which is formed must be of the highest quality from the standpoint of elasticity. The highest grade cake flours are those forming the least amount of gluten, but they must form enough gluten to hold the finished cake together and to form the final cellular structure. Flours for high-sugar cakes have a protein content of 7.5 to 8.5%; flours for the heavier cakes, 8.5 to 9.5%; and cookie flours, 8.0 to 9.0%.

(*c*) *Acidity or pH*

The *p*H or acidity of cake flour is an important factor in determining quality. It indicates whether proper bleaching and aging methods have been employed. The effect of bleaching is noticed principally in the performance of the flour as to volume, grain, texture, and color of the finished product. Chlorine (sometimes containing 1% nitrosyl chloride) is used to bleach and mature cake flours. Chlorine decreases the *p*H of a flour; cake flours should be bleached to a *p*H of from 5.0 to 5.2. Chlorine also makes the starch somewhat more soluble and causes dispersion of the gluten, thus reducing the doughing tendency of the flour.

Cookie flours should not be bleached with chlorine. Thus, they have more tendency to form gluten than do cake flours. They can be bleached with an agent, like nitrogen dioxide, which does not affect the gluten.

(*d*) *Granulation*

Flour granulation is of importance in producing quality cake, especially in producing good texture. The more uniform the flour granulation, the more uniform is the texture. Granulation also affects the volume and grain of the finished cake. It is not so important in cookie flour.

Many workers believe that cake flours should be as fine as economically feasible.

Current practice is to finish cake flours through 14 XX bolting cloth. Cake flours are much more finely ground than bread flours.

4. Creaming and Emulsifying Power of Batters

The gluten that is developed from the flour, as mentioned above, is not the only factor that affects the amount of gas retained by the cake batter. The emulsifying power of this batter is very important, and to a great extent depends upon the shortening and eggs. Many workers have attributed the ability of the cake batter to retain gas directly to the creaming power of the shortening. Today, emulsifying power seems to be stressed more; but it must be admitted that almost all shortenings having high emulsifying power also have high creaming power. The best shortenings for this purpose are the all-hydrogenated vegetable oils, referred to as hydrogenated shortenings. Those to be used especially for modern types of high-sugar cake have added to them emulsifying agents which in most cases are monoglycerides and diglycerides. Although plain hydrogenated shortening has excellent emulsifying properties, the special shortening with the emulsifying agent added is still better.

There is no doubt that true emulsification is of vital importance in modern cake production. Emulsifying shortenings actually make possible adding more moisture-retaining agents, such as sugar and sirups, and more ingredients containing moisture, such as eggs and milk, to the batter, thus producing a softer cake with better keeping qualities.

For cookies, crackers, and pie dough, most workers agree that it is neither creaming power nor emulsifying power which is of importance, but shortening power. This is described in the literature as true lubrication. Thus, cookies, crackers, or pie doughs containing shortening with the greatest shortening power will be the most tender, that is, they will break with the least effort. Lard and hydrogenated shortening are the best fats for this purpose.

5. Keeping Quality, Flavor, and *p*H

There has been a great deal of experimental work to determine the optimum *p*H for different cakes. In general, cakes show specific tendencies as they are varied from the acid to the alkaline side of the *p*H scale. On the acid side of the scale, a cake is lighter in color, more dense, and has better keeping qualities. On the alkaline side of the scale, the color of the cake is darker, it is more open and coarse, and the keeping qualities are poorer. It is thus necessary to arrive at a definite *p*H value which will give the optimum balance of all these factors. The *p*H values for different cakes recommended in the accompanying table are a result of the work of various investigators. The question mark placed after chocolate cake indicates that a great variety of cakes may be classed as chocolate cake. Cocoa or chocolate cakes having a *p*H range from 5 to 6 will have a cinnamon color; from 6 to 7, brown; from 7 to 7.5, mahogany; and from 7.5 to 8, a red-mahogany. Many bakers strive to obtain this reddish cast in cocoa cakes and add so much soda to obtain this that the *p*H is above 8.

Angel food	5.2–5.6
Chocolate	7 2–7.6?
Devil's food	7.5–8 0
Fruit cake	4 4–5.0
Pound	6 6–7.1
Sponge	7.3-7 6
White layer	7.1–7 4
Yellow layer	6.7–7.1

In general, the flavor of cakes is more desirable and added flavors are more pronounced if the cake is slightly acid than if it is neutral or alkaline; devil's food and chocolate cakes are exceptions.

The stability of the creamed emulsion of a cake is affected by *p*H. Various investigators have demonstrated that, when using hydrogenated shortening, the most stable emulsion is formed on the acid side of the *p*H scale. This means less chance for batter curdling. The commercial baker can make his creamed emulsion acid by the addition of an acid salt like cream of tartar or calcium acid phosphate. This is demonstrated by the fact that a pound cake batter that is slightly acid is much more effective in holding up raisins or other fruits than is the same batter when neutral. Too much acid, however, must not be used because it makes the cake too tender and too difficult to handle in production.

6. Balancing Cake Formulas

In order to obtain the proper state of emulsification in cake batters, and to produce batters that have the properties contributing to a satisfactory finished cake, the baker resorts to the practical method of using general rules that have been found to be rather reliable. These usually lead to a balanced formula having the proper qualities and proportions of essential ingredients for the making of quality cake. It must be remembered, however, that the rules given below lead to an approximate balanced formula; and the final formula must be reached by trial-and-error methods. The rules, therefore, are only guides. The fundamentals, however, are valuable to the baker as well as to the bakery technician in the development of new cakes and new processes.

The type of cake to be manufactured is to be considered. In general, there are two types: the low-sugar content cakes; and the high-sugar content cakes. (The latter containing the higher percentages of sugar (based on flour as 100%) are more popular.) In order to produce the former, the following rules should be observed:

(*1*) The percentage of sugar should be slightly less than the percentage of flour.
(*2*) The percentage of fat should be less than the percentage of eggs.
(*3*) The percentage of moisture, milk, and eggs should exceed the percentage of sugar.

If the second type of cake is to be produced, the following rules should be observed:

(*1*) The percentage of sugar should be more than the percentage of flour.
(*2*) The percentage of fat should be less than the percentage of eggs.
(*3*) The percentage of moisture, milk, and eggs should exceed the percentage of sugar

It should be noted that rules (*2*) and (*3*) are identical in both cases. Thus, the percentage of sugar in relation to the percentage of flour to a great extent controls the type of cake.

The balancing of cake formulas is not only a matter of balancing the ingredients so that a stable emulsion that will retain gas is formed, but is also a matter of balancing the ingredients that contribute toughness to the cake against the ingredients contributing to tenderness, and of balancing the ingredients that contribute moisture against those contributing to dryness. A suitable balance of these factors is important in order to produce a cake of proper tenderness and moistness.

Flour, milk solids, and egg solids are tougheners. Sugars, sugar sirups, shortening, fat of egg yolk, and leavening agents are tenderizers. All ingredients containing large quantities of water, such as sirups, liquid milk, eggs, etc., are moisteners; while flour, milk solids, and cocoa are driers.

If the rules for sugar exceeding flour are followed, cakes with good keeping qualities, moistness, tenderness, and good flavor will be produced.

7. Methods of Cake Mixing

The rules for balancing formulas are influenced to some extent by the method of mixing. The mixing methods include:

(*1*) The creaming method, in which the sugar is first creamed with the shortening.

(*2*) The blending method, in which the flour and shortening are blended and the other ingredients then mixed in.

(*3*) The method in which all the ingredients are placed in the mixing bowl and mixed.

When large quantities of moisture, eggs, milk, and sugar are used, the blending method generally works best. When smaller quantities of moisture, eggs, milk, and sugar are used, the creaming method is generally preferred. In most cases, method (*3*) is inferior to the other two, as it does not allow as good an incorporation of air.

Thus, the method of mixing used depends upon the type of ingredients used, as, for example, whether the milk is liquid or dry; whether the sugar is totally granulated or whether it is a mixture of granulated sugar and sirups; whether the shortening is regular hydrogenated vegetable oil or whether it has emulsifiers added to it; whether the eggs are fresh eggs, frozen eggs, or dry eggs, etc.

8. Basic Cake Formulas

Basically, there are only four different types of cake. These are pound, layer, sponge, and angel food. The pound, layer, and sponge cakes can be made with the amount of sugar less than that of flour or the amount of sugar more than that of flour. The basic formulas are given in Table 148.

All other cakes are variations of these basic formulas obtained by the addition of ingredients such as cocoa, chocolate, bananas, pumpkins, fruit, nuts, flavors, jellies, etc. Special modifications are necessary at times, for example, the use of additional

TABLE 148

TYPICAL CAKE FORMULAS

Figures Represent Per Cent on Basis of Flour 100%

Ingredient	Original pound	Modern high-sugar pound	Low-sugar layer	Modern high-sugar layer	Modern sponge	Modern angel food
Flour	100	100	100	100	100	100
Butter and/or shortening	100	60–70	35–45	45–55	0	0
Sugar	100	120–130	80–100	120–140	110–120	300
Eggs (whole)	100	70–75	40–50 or	55–70 or	65–75	0
Eggs (whites)	0	0	40–50	55–70	0	300
Eggs (yolks)	0	0	0	0	45–55	0
Milk (liquid)	0	55–60	50–60	90–95	0	0
Salt	0	3.0–3.5	2.5–3.0	3.0–4.0	2.0–3.0	4.0–5.0
Baking powder	0	0–2	2–4	4–6	2–3	0
Cream of tartar	0	0	0	0	0	4–5

moisture to take care of the absorption of cocoa, the adjustment of the pH to take care of the acidity of natural fruits, etc.

VII. DEVELOPMENT OF STALENESS

The development of so-called "staleness" in bakery products such as bread, yeast-raised sweet goods, and cake is rapid. Thus, because all these products are very perishable they must be placed in the consumer's hands while still fresh. If they become too old, their general attractiveness and freshness will be lost.

From the practical point of view, the most important cause of staleness is loss of moisture, which modern wrappers will prevent to a significant degree for a period of several days.

Bakery products also become stale due to a change in the form of the starch, which there is no practical way of preventing. This change occurs whether the product is allowed to lose moisture or whether it is packed in a moistureproof package. The subject has been thoroughly reviewed by Alsberg[20] and by Cathcart.[21] Much research has been carried out on this problem of staleness. The most feasible means of preventing the change in the form of starch from the fresh to the stale state is by freezing the product at a low temperature.[22] Preliminary experiments show that the method is applicable to cakes and pies.[23] The success of the method depends to a great extent of the product's being wrapped in such a manner that little or no moisture is lost. Best results are obtained when the products are hermetically sealed: in fact, bread can be kept at freezing temperatures in commercially fresh condition for approximately one year when so sealed.[24]

---

[20] C. L. Alsberg, *Wheat Studies Food Research Inst.*, **12**, 221 (1936).

[21] W. H. Cathcart, *Cereal Chem.*, **17**, 100 (1940).

[22] W. H. Cathcart and S. V. Luber, *Ind. Eng. Chem.*, **31**, 362 (1939).

[23] W. H. Cathcart, and W. Pushnik, Am. Bakers' Assoc., *Bull.* **2**, 443 (1939).

[24] W. H. Cathcart, *Cereal Chem.*, **18**, 771 (1941).

1. Physical Changes during Staling

In wrapped bread, the crust as well as the crumb shows certain changes during staling, *i. e.*, it loses its crispness.

The various changes that take place during staling of bread are as follows:

(*1*) The crust becomes soft and leathery.
(*2*) The crumb becomes hard and crumbly.
(*3*) The amount of soluble starch decreases.
(*4*) The crumb dries out.

Most of the work that has been reported has been carried out on bread. However, practically all of these changes take place in sweet yeast-raised goods and cakes as well as in bread. It is interesting to note that chemical analyses show no appreciable difference between fresh and stale bread. No difference in any factor is noted until the bread shows bacterial or mold growth.

2. Crumb Staling in Bread

As early as 1852, Boussingault showed that bread would stale when kept in a perfectly closed container. He also showed that the bread could be brought back to freshness by heating to 60° C. or higher. The mystery of staling has thus been known for many years and yet has not been solved. The change that takes place in the form of the starch, as Boussingault recognized, can be followed by these methods:

(*1*) Increase of crumbliness and hardness of the crumb.
(*2*) Decrease of swelling of the crumb in water
(*3*) Decrease in amount of soluble starch.
(*4*) Change in x-ray pattern.

The change in the form of the starch is probably physical, a point which, however, has never been definitely settled. If it is physical, there is no need to look for a catalytic agent to prevent the change; to date, no such catalyst has been found. Katz[25] considers the change a reversible equilibrium dependent upon temperature for its direction of reaction. Above 60° C., the fresh state is the stable form of the starch; below 50° F., the stale state is the stable form. However, below freezing the rate of staling is decreased because the bread freezes solid and all reactions are retarded in the solid state.

3. Methods of Measuring Staleness

There are many ways of following the changes in the form of starch in bread and bakery products. Since this change takes place very rapidly and is nearly complete in 20 hours, it is the feeling of the practical scientist that, for bakery products, it is to be considered in combination with the change in the hardness in the crumb. Thus, machines for measuring the softness of the crumb, called compressibility machines, or compressometers,[25a, 26] have been developed (Fig. 60). Even after considering all of the tests described in the literature, the author is inclined to feel that none is a very good comparison with consumer reaction, and that the only way really to tell when a product is stale from the consumer's point of view is by organoleptic methods.

---

[25] J. R. Katz, chapter in *A Comprehensive Survey of Starch Chemistry.* Vol. 1, Chemical Catalog Co., New York, 1928, p. 100.

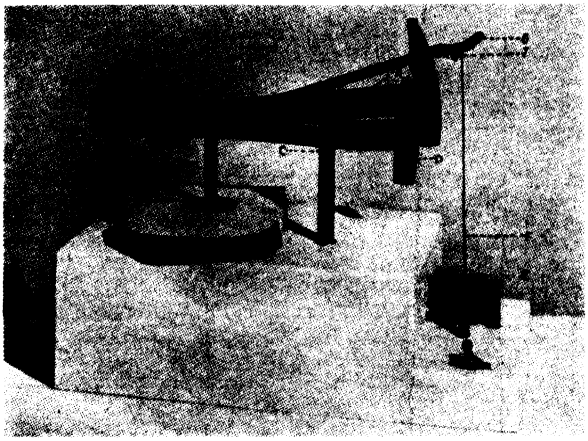


Fig. 60.—The latest type of compressometer for measuring the softness of bread crumbs.[25a] Drum *E* is operated by motor and depresses lever *G* at a uniform rate. After lever *G* has reached a predetermined point on scale *J* corresponding to the application of a certain stress, the corresponding depression or strain on scale *D* is read. The compressibility of the bread is recorded as the average of the readings for all the slices in one or more loaves of bread. (Courtesy of The American Association of Cereal Chemists.)

4. Producing Bread and Sweet Goods with Good Keeping Quality

The two most effective methods for delaying crumb staling, heat and cold, are not practical at the present time. Examination of the data for retarding the staling of crust and crumb indicates that a palatable product of good keeping qualities can be produced by:

(*1*) Using flour with good baking quality.

(*2*) Using liberal amounts of milk, shortening, sugar (preferably sirup), and eggs (in products where they are used).

(*3*) Using normal amounts of salt and yeast.

(*4*) Using fairly high concentrations of diastatic enzymes, and perhaps some moisture-retaining agent, such as gelatinized starch, dextrins, etc.

(*5*) Making a medium-slack dough by high-speed mixing.

(*6*) Proper fermentation, handling, and baking.

(*7*) Rapid cooling, and wrapping after adequate cooling.

[25a] *Cereal Chem.*, **17**, 613 (1940).

[26] W. Platt, *Cereal Chem.*, **7**, 1 (1930).

5. Cake Staling

The staling of cake is entirely similar to that of yeast-raised products. However, in cake, moisture is a much more important factor than the change in the form of the starch. Unpublished observations of the author show that the most important factor in the staleness of cake as it reaches the consumer is the length of time the cake is allowed to stand, before wrapping, after it has come out of the oven. Of second importance is the length of the baking time. An incorrect, long baking time produces stale cake at the mouth of the oven.

Cake that is made with good sound ingredients, that is not overbaked and is cooled quickly and wrapped immediately in a moistureproof wrapper will remain fresh for 2 to 3 days. Cakes that are slightly acid will keep better than cakes that are neutral or alkaline. They also remain more palatable for a longer period. Angel food, for example, is one of the best keeping cakes; its *p*H is 5.5. Devil's food is one of the poorest keeping cakes; its *p*H is approximately 8.0.

It should also be pointed out that richer mixes tend to increase the life of cake. Too rich mixes should not be employed, however, especially in regard to sugar, for cakes containing more than 125% sugar on the basis of the flour begin to develop a dryness and strawlike character. From the author's experience, mixes containing 140% sugar on the basis of flour are generally inferior to those containing 125%.

VIII. NUTRITIVE PROPERTIES OF BAKERS' PRODUCTS

Bakers make a wide variety of products varying widely in composition and, consequently, in nutritive properties. However, in general, all bakery products have relatively large quantities of carbohydrates and significant quantities of fat, and are thus good energy foods. For example, bread furnishes about 1200 cal. per lb. Cake will furnish somewhat more, depending upon the type of cake: Angel food contributes about 1200 cal. per lb., and pound cake and doughnuts about 2000 cal. per lb., with the other cakes being of intermediate value. The compositions of various bakery products are given in Table 149. The values are taken from a report by Chatfield and Adams.[27]

Dry bakery products, such as crackers and cookies, give somewhat more calories per pound than bread, dependent mainly on the lesser moisture content. Most bakery products are made with added milk, sugar or sweetening agent, and shortening or butter. In addition, cakes and sweet yeast-raised goods have eggs added. These ingredients, in addition to the flour, mineral salts, etc., give bakery products a value over and above their calorific content.

Space is not available to enter into a discussion of the details of the nutritive quality of every product the baker makes. Since, from the standpoint of quantity and of a universally eaten food, white bread is the baking industry's important item, the following nutritive details will be limited to white bread. As made today it contains more sugar, more shortening, more milk, and, in general, more vitamins and minerals than white bread made 10 to 20 years ago. Because of these changes in ingredients, today's white bread contains approximately 9% high-quality protein, 3.5 to 4% fat,[28] and retains its 1225 cal. per lb. with only an average of 35.5% moisture.[29] This bread contains, on the average, 0.08% calcium,[30] with a calcium-phosphorus ratio that is

---

[27] C. Chatfield and G. Adams, U. S. Dept. Agr., *Circ.* **549** (1940).

TABLE 149

PROXIMATE COMPOSITION OF AMERICAN FOOD MATERIALS

Bakery product	Nature of sample	Water, %	Protein, %	Fat, %	Ash, %	Carbohydrates, %		Fuel value, cal.	
						Total	Fiber	Per 100 g.	Per lb.
Breads:									
White commercial[a]	Containing some milk solids	35.9	8.5	2.0	1.3	52.3	0.3	261	1185
Vienna	No milk or shortening	34.1	8.4	1.0	1.3	55.2	0.3	263	1195
Rolls	......	29.4	8.2	6.1	2.2	54.1	0.2	304	1380
Raisin	......	33.0	9.0	3.0	2.0	53.0	0.8	275	1245
Whole wheat	Made with water	37.0	9.0	3.0	2.0	49.0	1.0	259	1175
Whole wheat	Made with some milk	37.0	9.5	3.5	2.0	48.0	1.0	262	1185
Gluten	.....	39.1	25.1	3.8	1.8	30.2	0.3	255	1160
Boston brown	......	48.4	4.9	2.5	3.0	41.2	0.5	207	940
Biscuits	......	31.0	7.3	13.0	2.5	46.2	0.2	331	1500
Crackers:									
Water, shortened	......	7.0	10.1	4.8	1.3	76.8	0.5	391	1775
Soda, plain	......	5.7	9.6	9.6	2.4	72.7	0.2	416	1885
Saltine	......	4.6	9.2	11.8	3.3	71.1	0.4	427	1940
Graham	......	5.5	8.0	10.0	2.2	74.3	0.8	419	1900
Cakes:									
Angel	......	31.6	8.4	0.3	1.0	58.7	0.0	271	1230
Fruit, dark	......	22.9	5.2	13.8	2.2	55.9	1.2	369	1670
Plain	......	26.8	6.4	8.2	1.6	57.0	0.1	327	1485
Plain, frosted	......	25.2	5.2	6.2	1.3	62.1	0.1	325	1475
Pound	......	19.3	7.1	23.5	0.8	49.3	0.1	437	1985
Sponge	......	31.8	7.9	5.0	0.9	54.4	0.2	294	1335
Cookies:									
Crisp, thin, rich	Any flavor, including chocolate	3.0	7.8	18.0	1.4	69.8	0.2	472	2145
Soft, thick	Any flavor, including chocolate	7.8	6.8	10.5	1.9	73.0	0.2	414	1875
Sandwich type, commercial	Any flavor, including chocolate	2.3	5.0	19.6	0.9	72.2	0.3	485	2200
Coconut bars	......	4.0	6.2	16.7	1.5	71.6	...	462	2095
Fig bars	......	13.8	4.2	4.8	1.4	75.8	1.7	363	1645
Macaroons	......	10.5	6.3	16.9	0.9	65.4	1.1	439	1990
Molasses	......	5.5	6.4	8.9	2.5	76.7	0.4	412	1870
Peanut	......	2.6	14.0	27.5	2.4	53.5	0.8	518	2345
Vanilla wafers	......	5.6	6.1	14.9	1.0	72.4	0.3	448	2035
Doughnuts	......	18.7	6.6	21.0	1.0	52.7	0.2	426	1935

[a] According to the author's work the following percentages would be better averages for commercial white bread: water, 35.5; protein, 9.0; fat, 3.8; ash, 1.8; total carbohydrate, 50.0; and calories per lb., 1225.0.

---

[28] W. H. Cathcart, unpublished observations.

[29] W. H. Cathcart, *Proc. Inst. Food Tech.*, **1940,** p. 51.

[30] W. W. Prouty and W. H. Cathcart, *J. Nutrition*, **18,** 217 (1939).

approximately 1:1.[31] In addition, the recent syntheses of the B vitamins have made it possible for bakers to put back into their white bread certain of the nutritive factors removed from the wheat by milling. A national program has been developed in this regard,[32, 33] and, as a result, since August, 1942, 65% of all the white bread sold in the United States is "enriched" with thiamine, niacin, and iron, according to the schedule given in Table 150. White bread can be enriched by the addition of synthetic vitamins and minerals or partially by special methods of milling whereby these nutrients are retained in the flour.

TABLE 150

PROPOSED STANDARDS FOR ENRICHED BREAD

(According to Committee on Foods and Nutrition, National Research Council)

Ingredients	Milligrams per pound	
	Minimum	Maximum
Required:		
Thiamine	1 0	4.0
Niacin	4 0	16.0
Iron	4 0	16.0
Optional:		
Riboflavin	0 8	3 2
Calcium	333 0	1333 0
Vitamin D (U. S. P. Units)	160.0	640 0

TABLE 150*a*

PROPOSED STANDARDS FOR ENRICHED BREAD

(According to *Federal Register*, 8, No. 152, Aug. 3, 1943)

Ingredients	Milligrams per pound of bread	
	Minimum	Maximum
Required		
Thiamine	1 1	1 8
Niacin	10 0	15.0
Riboflavin	0.7	1.6
Iron	8 0	12.5
Optional:		
Calcium	300	800
Vitamin D (U. S. P. units)	150	750

It is now mandatory, according to Food Distribution Order No. 1, for all bakers to enrich all yeast-raised products and doughnuts (yeast-raised or baking powder-raised) to the extent of the white flour present. Addition of vitamins and minerals to white bread, rolls, and buns became effective October 1, 1943; for all other bakery products, probably July 1, 1944. The enrichment standards for white flour given in Table 140 (page 666) result in at least the minimum values for white bread given in Table 150*a*.

---

[31] W. W. Prouty and W. H. Cathcart, *J. Am. Dietet. Assoc.*, **17**, 450 (1941).

[32] J. A. Tobey and W H Cathcart, *Ind Eng. Chem.*, **33**, 713 (1941).

[33] W. H Cathcart, presentation before the Semi-Annual Meeting of Am. Chem. Soc., Buffalo, N. Y., 1942.

Thus, the white bread produced is approximately equivalent to whole wheat bread in thiamine, riboflavin, niacin, and iron. Experiments indicate that modern white bread made with milk, when enriched according to the present requirements, enables the rat to grow as well as on whole wheat bread.[34]

Thus, modern enriched white bread is a loaf of bread with which food faddists can find little fault, one that is approximately equivalent to whole wheat bread in its nutritive qualities, and a loaf that the baking industry is giving to the nation as a whole at no additional cost. There is no doubt that it is a great contribution to the health and welfare of the American people.

IX. MICROBIOLOGY OF BAKERY PRODUCTS

The baking industry has both desirable and undesirable types of microorganisms with which to contend. The problem is the inhibition of the growth of the undesirable types without retarding too greatly the desirable types. In some products, such as cakes and pastries, the problem is one of inhibiting the growth of all microorganisms. The baker is mainly interested in the desirable types of microorganism growth in all yeast-leavened bakery products, the most important organism being that of yeast.

Yeasts which are being supplied to bakers today for leavening doughs are of the *Saccharomyces cerevisiae* species. Some strains within this species are stronger and more valuable for baking purposes than others. Yeast companies supply desirable strains of this species in a "pure" form, so that the baker has a reliable source of constant and uniform yeast. "Wild yeasts" (the baker considers all species and strains other than those inoculated into the dough batch as "wild") must be classified as undesirable in fermentation, for they are likely to produce irregularities in the fermentation as well as undesirable products. Certain yeasts classified by the baker as "wild," which have been found in doughs, are *S. ellipsoideus* and *Mycoderma cerevisiae.* There are many others. Little trouble is experienced with wild yeast today because rather high inoculations are made with pure strains of compressed yeast and fermentation conditions are generally well controlled.

Although a much debated question, it is very probable that the growth of many types of bacteria is also desirable during yeast fermentation. Foı example, a small growth of lactic, acetic, propionic, and butyric acid producers will form these acids and enhance the flavor of the product, although the presence of these acids in any quantity would be undesirable. Other bacteria which produce a variety of products are perhaps desirable to a small extent. A baker can limit the production of acetic, lactic, propionic, or butyric acid by controlling the temperature and/or the duration of the fermentation. At the optimum temperature of fermentation in the bakery, 78 to 80° F., acid-producing bacteria do not grow well. Sometimes bakers who do not have controlled fermentation conditions have trouble with sour bread's being produced when a long fermentation period is used. This is simply due to overgrowing of the yeast by acid-producing bacteria. Of course, this depends on the number of acid-producing bacteria originally present. Sour bread is not a common occurrence today, especially when the sponge and dough process is used.

---

[34] R. F. Light and C. N. Frey, *Cereal Chem.*, 20, 645 (1943).

Large quantities of acetic and lactic acid produced by bacteria would be desirable when special sour doughs are made, as for example in the making of sour rye bread.

Of course, it is understood that microorganisms producing large quantities of gas, diastase, and/or protease are undesirable. These organisms will cause variations in the fermentation and resulting products. Some persons in the industry have at times attributed holes in bread to gas-forming bacteria. *Escherichia coli* and *Clostridium welchii* form gas in doughs and have been used experimentally as leavening agents for bread.[35]

Bacterial salt-rising fermentation would be undesirable except where "salt-rising" bread is being made. Salt-rising bacteria work best at a *p*H of 6 to 8, at high temperatures, and when milk is present in the formula. Thus, under normal fermentation conditions there is no interference with normal bread production.

Isolated cases of undesirable bacterial growth, besides those mentioned above, do occur during the yeast fermentation of a dough. These are due to slime-forming organisms such as those producing "rope." However, in general, these types of bacterial growth do not show up in the product unless the bacterial spores survive the baking process and until the bacteria have had an opportunity to multiply after the product is baked. Bakery products which occasionally show "ropy" or slimy developments are generally old and have been stored under conditions ideal for bacterial growth. These conditions are: available food, which is supplied by the bakery product; warm temperatures; sufficient moisture; proper conditions of hydrogen-ion concentrations, etc.

1. Nature of "Rope" Infection

The condition in bread known as "rope" is due to a spore-forming bacterium, *Bacillus mesentericus*. Lloyd and McCrea[36] have shown that the spores of *B. mesentericus* species can resist the temperature of boiling water for a period of 30 minutes to 6 hours. Thus, since the temperature of the interior of baked products (such as biscuits, muffins, cakes, and breads) during baking does not exceed 100° C.,[37] the "rope" spores at the interior will not be killed during baking. If any are present in the dough they will thus pass through the baking operation and growth will start in or near the center of the loaf. Although "rope" formation is generally confined to bread, it can develop in cakes and other bakery products, but this development is extremely unlikely because of the lower moisture contents of bakery products other than bread.

"Rope" is a warm weather disease, developing most rapidly at 98 to 104° F. The properties of bread that have become "ropy" are as follows: an overripe cantaloupe or melon odor; discoloration of the crumb—it may vary from a yellow to a brownish hue; softening of the crumb and formation of thin, gelatinous threads when drawn out. The first stages of "rope" show only the odor. The next stage to develop is the discoloration. Last of all, the crumb begins to soften. These changes are illustrated in Figs. 61 and 62. However, it must be remembered that "rope" must be well advanced before the odor is determinable. The softening of the crumb is undoubtedly due to the action of diastatic and proteolytic enzymes, thus enabling the crumb to be drawn out in strands. Fisher and Halton[38] state that "ropiness" is due to the formation of gum and sugar (probably mannite) through the decomposition of starch by amylase, in which these organisms are rich. They point out that the initial growth of the organism in bread is upon the soluble phosphate, soluble nitrogen, and sugar. After these are exhausted, the organism attacks the loaf protein, a decomposition accompanied by the characteristic odor described above.

---

[35] F. W. Tanner, *Bacteriology*. Wiley, New York, 1928.

[36] D. J. Lloyd and E. D. McCrea, *Bull.* **48**, Rept. to Food (War) Comm., Roy. Soc. (1918).

[37] R. A. Barackman and R. N. Bell, *Cereal Chem.*, **15**, 841 (1938).

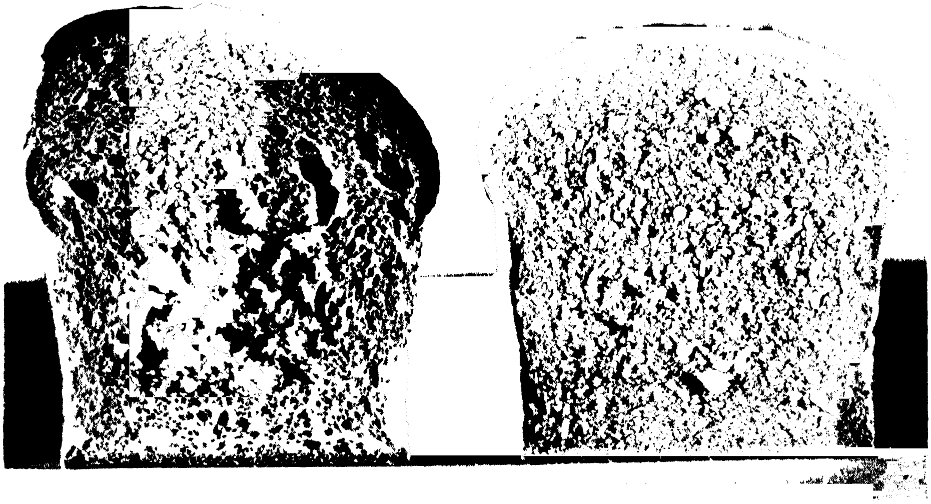


Fig 61 —Ropy bread The loaf on the left is "ropy," showing the disintegration of the crumb, the loaf on the right is normal (Courtesy of The Fleischmann Laboratories)

It is the opinion of Eckles[39] and others that no danger to health is forthcoming from eating bread contaminated with organisms producing this "ropy" condition. The author was also unable to find any evidence showing that "ropy" bread had ever made anyone ill.

2. Sources of the "Rope" Infection Organism

From a review of the literature it appears that the infection is caused by several strains of the *B. mesentericus* species.

---

[38] E. A. Fisher and P Halton, *Cereal Chem* , 5, 192 (1928).

[39] C. H. Eckles, *Proc. Iowa Acad Sci* , 7, 165 (1899)

B. mesentericus species are widespread in nature, and are found in the soil. They are very common on potatoes, and have been referred to as the "potato bacilli." Potato flour and potato products are likely to be a source of these spores unless carefully controlled. Slime-forming bacteria get on the grain and thus into flour, malt extract, and yeast worts, and then into baker's bread. It is said that they spread from cattle into milk, fruit into wine, and grain into beer.[40]

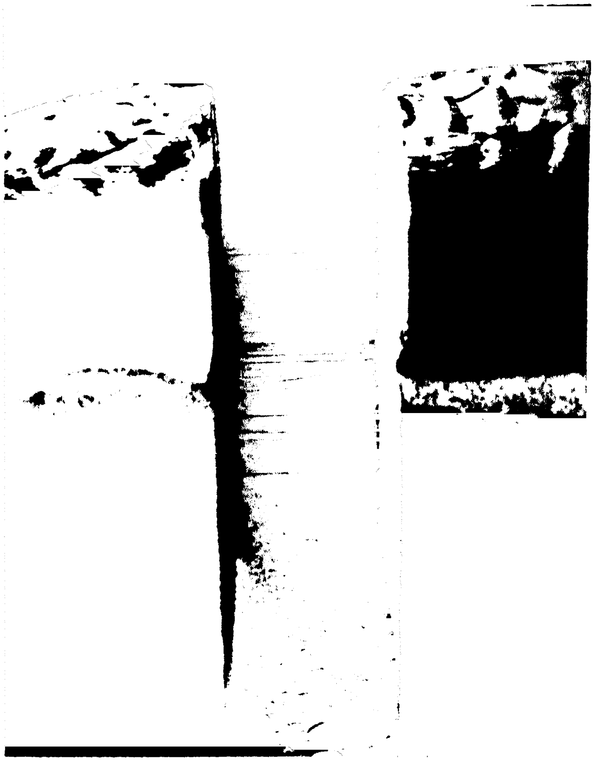


Fig. 62 – Ropy bread. A loaf of "ropy" bread cut and drawn directly out. The strands of disintegrated crumb are plainly visible The fact that "ropy" bread can be drawn out in such a manner probably accounts for the name "rope" These strands appear at times in cakes and cookies from the use of large quantities of sirup. In these cases, the odor of "rope" is absent. (Courtesy of The Fleischmann Laboratories)

The literature indicates that flour (all kinds of flour, high and low extractions from wheat, rye, barley, and other cereal grains) is the chief carrier of the organisms causing "rope" in bread. However, it has been pointed out[41] that, in the United States, other ingredients than flour are the main sources of "rope" infection. Hoffman, Schweitzer, and Dalby[41] state that in the period between 1929 to 1937, no "rope" spores were found in any flour sample examined. They did not test any foreign flours. They also state that improved methods of harvesting and milling of American grains appear to have had a beneficial effect on removing "rope" bacilli from the flour. Counts are reported of "rope" bacilli in yeast up to 20,000 per gram. The fact that "rope" can exist in yeast explains some of the mysteries of its appearance in bread. These workers also report that yeast companies were able to reduce the count in the yeast, once informed of it, and have been able to keep the count down to less than 100 per gram. Although malt extracts are also dangerous potential sources of "rope" infection, extracts can be purchased which are relatively free from spores. Molasses, milk, and sugar at times show small numbers of spores.

---

[40] E. A. Fisher and P. Halton, *Cereal Chem.*, **5**, 192 (1928).

[41] C. Hoffman, T. R. Schweitzer, and G. Dalby, *Ind. Eng. Chem.*, **29**, 464 (1937).

Except from the slicing machine, there is no danger of "rope" contamination after the bread leaves the oven. If the slicing blades are contaminated with "rope" spores, they will carry the spores into the center of the slices and thus contaminate the bread.

3. Detection of "Rope" in Ingredients and Finished Products

The most promising method for the estimation of the bacterial spores in any ingredient or product is the dilution method[42] which is based on the fact that "rope" bacilli form a pellicle or surface growth on standard bouillon. The method does not distinguish between "rope" spores and other spore-forming bacteria giving a surface growth. Tests show, however, that only rarely do the pellicle-forming spores prove to be other than "rope" spores. The test can be made more conclusive by inoculating pieces of sterile bread in test tubes with the pellicle-forming culture, incubating the tubes, and then observing the bread.

4. The Effect of Acids and Acid Salts

The development of "rope" organisms is inhibited by an acid medium. Watkins[43] was one of the first workers to study the influence of temperature, moisture, and reaction on the development of the infection, and advises the use of acids in its control. However, it is often difficult to produce bread of good quality and at the same time obtain the required *p*H of approximately 5 for retarding the development of "rope."

Kirby, Atkin, and Frey[44] have shown that vinegar and monocalcium phosphate are more effective in inhibiting "rope" growth than other acid ingredients (quantities given in Table 151). They found that it is not necessary to reduce the *p*H of bread to as great an extent with these two ingredients as it is with other acid-acting materials; thus, they do not have as much effect on the resulting bread quality. These investigators have shown that the specific anti-"rope" action, other than that due to mere hydrogen-ion concentration in the case of acetic acid, is due to undissociated acetic acid molecules.

[42] A. J. Amos and D. W. Kent-Jones, *Analyst*, **56**, 572 (1931)

[43] E. J. Watkins, *J. Soc. Chem. Ind.*, **25**, 350 (1906).

[44] G. W. Kirby, L. Atkin, and C. N. Frey, *Food Industries*, **8**, 450 (Sept., 1936)

Even if a dough has been properly treated with acid it may not inhibit "rope" in the bread if proper cooling and storage are neglected. Furthermore, it is not always safe simply to add, without *p*H measurements, a recommended amount of a certain acid or acid salt for the prevention of "rope." This is because some ingredients which the baker uses, especially milk, affect the *p*H of the finished product, that is, exert a buffer effect on the acidity. A loaf of bread with 3% milk solids having a *p*H of 5.4 will have a *p*H of 5.7 when made with 12% milk solids. Thus, more acid must be added to a dough containing a high percentage of milk solids than one with little or no milk solids, in order to bring them both to the same *p*H value.

TABLE 151

ACIDS, ACID SALTS, AND PROPIONATES RECOMMENDED FOR INHIBITING "ROPE" IN BREAD

Acid or acid salt	Quantity per bbl. (196 lbs.) flour	Per cent on basis of flour as 100%
Vinegar (100 grain, about 10% acetic acid)	1.0- 1.6 pts.	0.5- 0.8
Monocalcium phosphate	13.0-22.0 oz.	0.4- 0.7
Potassium bitartrate (cream of tartar)	15.7 oz.	0.5
Lactic acid (70%)	11.0 oz.	0.35[a]
Calcium propionate, or sodium propionate	4.0-10.0 oz.	0.13-0.30

[a] This quantity is necessary to inhibit "rope," but Morison and Collatz have found it to harm bread quality with some flours. Some other workers, however, have recommended it.

Although larger quantities of the acids or acid salts listed in Table 151 would, undoubtedly, completely inhibit the growth of "rope," larger quantities than listed will have a harmful effect upon bread quality.

The most that can be hoped for in the acid treatment of doughs is that the development of "rope" will be delayed long enough so that it does not become apparent before the bread is consumed.

5. The Effect of Propionates[45]

Propionates are available commercially which are effective in delaying the development of "rope" in bread and other products. The commercial preparation of calcium or sodium propionate is recommended in a minimum quantity of 0.125% and in a maximum quantity of 0.30%, on the basis of flour as 100%, for the elimination of "rope" in bread. (These quantities are also recommended for eliminating mold in bread.) The maximum quantity given is recommended for dark breads. The effectiveness of propionates depends to some extent on the *p*H of the product; the slight acidity of yeast-raised products, such as bread, is favorable to their action. However, in cakes, which are either neutral or alkaline, it is necessary to use larger quantities of propionates to have the same effect (used mainly, in cakes, to inhibit mold). Since larger quantities of calcium propionate than 0.3% may affect the flavor of the product, the manufacturers recommend for cake sodium propionate instead of the calcium salt. Another reason is that calcium propionate has a greater lowering effect on *p*H than sodium propionate. The quantities of the commercial preparation recommended for cakes are based on the total batter, and range from 2 oz. per 100 lbs. total batter for the more acid batters to 7 oz. per 100 lbs. for the more alkaline.

---

[45] Sodium diacetate has just been recently proposed as a "rope" and mold inhibitor. If tests show that it is effective for this purpose, it may be used in place of propionates.

6. Precautions for the Prevention of "Rope"

The first important factor in the prevention of "rope" is the removal of the cause for it; the second, removal of conditions (as far as possible) favorable to its growth; and lastly, the application of acids or propionates. Thus, the following precautions should be taken: All equipment, storerooms, cooling rooms, delivery trucks, trays, and, in fact, the entire bakery must be kept in a clean, sanitary condition; this may necessitate the generous use of germicides like hypochlorites and ultraviolet bactericidal lamps. It is very important that the ingredients be stored in a clean, cool, dry place to prevent bacteria and molds from growing in them. It is also important that stale bakery products are not brought back into the bakery, for they may be infected with "rope" and mold. Too much moisture in the product should be avoided—thus, bread should be thoroughly baked and completely cooled (an inside crumb temperature of 85° F. is desirable before wrapping). Bread should be cooled quickly and wrapped under sanitary conditions. During fermentation, temperatures should be controlled around 80° F., for higher temperatures may allow a dangerous increase in "rope" organisms if they are present in sufficient quantities. Stores should not be oversupplied with products; their bakery racks should be in a clean, cool, dry, well-ventilated place, not in a hot, damp spot, or "rope" and mold will make their appearance even before the product is purchased.

7. Quality Control

The baker should practice "quality control" in connection with the protection of his products from "rope" and also mold. All he needs to do is to save 3 or 4 loaves or other products, wrapped in the usual manner, from each day's baking, place them in an incubator at 95-100° F. or in a comparable place in the bakery and test one sample of each at various time intervals, for instance, at 24-, 48-, and 72-hr. intervals, for the development of infection. If the products pass the 72-hr. test successfully, they may be assumed to be satisfactory. If "rope" or mold develops within 48 hours, the baker should institute emergency "rope" and mold control measures. In this way he will know before the consumer of any trouble which may develop.

Ingredients should be checked constantly to make certain that they are not contaminated with or do not contain an excessive number of "rope" spores. Even though ingredients are acceptable with respect to spore count, the baker should still follow the above precautions for the prevention of "rope," or his products may still become "ropy."

8. Other Slime-Producing Bacteria

Eckles[46] and Amos and Kent-Jones[47], point out that bread inoculated with *Bacillus subtilis,* the "hay bacillus" (invariably found in hay), developed a slight yellow coloration and an odor somewhat resembling that from the growth of *B. mesentericus.* No stringiness was found, however. The author has noticed this condition in some loaves of bread; the cause was found to be the dried milk used, although the organisms were not identified. The sample of milk was tested for "rope" by the dilution method described above, and the number of spores found were less than 10 per gram. Eckles also states that *B. liodermos* will produce the slimy decomposition (however, of a lighter color) in sterile bread that is characteristic of bacilli belonging to the *B. mesentericus* species. The literature indicates that spores of *B. liodermos* will pass through the baking process. There is evidence that spores of *B. subtilis* will also do so. These organisms can be eliminated and controlled in a manner similar to that used for organisms producing "rope."

X. "BLEEDING" BREAD

"Bleeding" bread (sometimes called "red" or "bloody" bread, "wunderblut" and "blood rain"), although in modern times an uncommon abnormality in this country, has been experienced in the past, especially in other nations. It appears as spots (sometimes streaks) resembling drops of blood upon the surface of bread and other products. Some writers say that it usually occurs on the moist bread crumb. It is due to the growth of bacteria which form red pigments. Tanner[48] states that Harrison attributes this so-called "bloody bread" to the growth of *Serratia marcescens* (*B. prodigiosus*). This same organism has been known to cause the so-called "red milk."[48] It is agreed that it is harmless to the human body; and indications are that it can be controlled in the same way as "rope."

Another uncommon "bloody stain" of bread has been reported which has a different characteristic. It appears more often on dark bread and on the crumb rather than on the surface. The cause is said to be a certain microscopic fungus, *Oidium aurantiacum,* for which flour is reported to be the source.

XI. MOLD ON BAKERY PRODUCTS

There are various genera of molds which have been found on bakery products. Herter and Fornet[49] have published a rather complete study of the mold problem in bread. In reviewing the literature up to 1910, they conclude that molds belonging to the *Aspergillus, Rhizopus,* and *Penicillium* genera were most prevalent on bread. *Oöspora, Mucor,* and *Monilia* also were found. Prescott, Streider, and McClellan[50] found that *Rhizopus*, *Aspergillus*, and *Penicillium* were constantly present in the moldy breads which they studied.

---

[46] C. H. Eckles, *Proc. Iowa Acad Sci.*, **7**, 165 (1899).

[47] A. J. Amos and D. W. Kent-Jones, *Analyst*, **56**, 572 (1931).

[48] F. W. Tanner, *Bacteriology*. Wiley, New York, 1928.

[49] W. Herter and A. Fornet, *Centr. Bakt. Parasitenk.*, **A, II, 49**, 148 (1919).

[50] S. C. Prescott, J. W. Streider, and R. N. McClellan, *Baking Tech.*, **1**, 142, 188, 230, 282 (1922).

Several workers have concluded that no mold spores survive the bread-baking process. *Thus, if a baker employs a modern mixing technique, he may consider his products sterile with respect to mold when they come from the oven.* Mold infection, then, occurs after the product has been baked. Even though this is the case, the presence of molds in ingredients is undesirable because, if they have developed far enough, they will contribute a musty flavor to the resulting product.

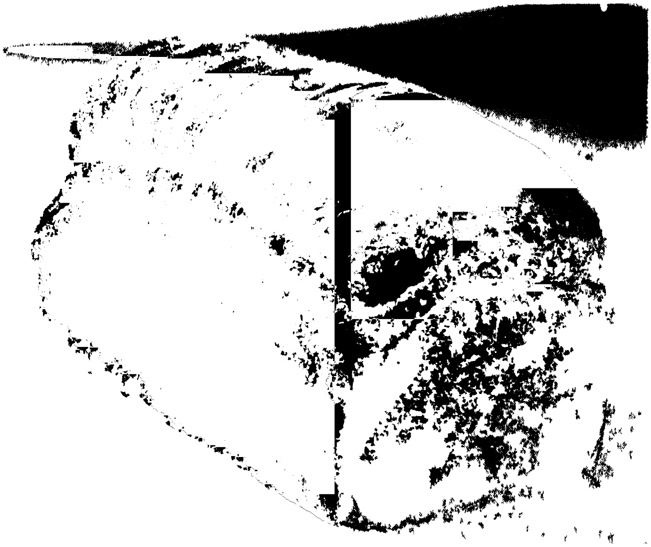


Fig. 63.—A loaf of moldy bread. (Courtesy of The Fleischmann Laboratories.)

In general, then, the control of mold is different from the control of "rope." One must protect against mold infection *after* the products are baked and against "rope" infection *before* the products are baked. As pointed out previously, once sufficient "rope" spores get into the ingredients or into the dough batch, great numbers will survive the baking temperature; and "ropiness" will develop in the baked product if the moisture, temperature, and acidity conditions are favorable. Mold spores get on the bread during the cooling, slicing, and wrapping processes, and will develop if conditions of moisture and temperature are favorable. (See Fig. 63).

It must be remembered that the problem of mold is not confined to bread, but includes cakes, pastries, and almost all bakery products.

1. Favorable Conditions for Mold Growth

The first requirement for mold growth is an available source of food and moisture, which is supplied by bakery products. The wrapping of modern bread, cakes, etc., causes to be built up around the products a high relative humidity favoring mold growth.

Warm temperatures (90–100° F.) favor the growth of most "bakery molds." However, *Penicillium* species grow well at lower temperatures.

Darkness is more favorable to mold growth than light, undoubtedly due in part to the ultraviolet radiation and also the dryness that always accompanies it.

It is interesting to note that molds grow under semianaerobic conditions and under aerobic conditions, although not as well, however, under the former. It may be said that most species require an ample supply of air or oxygen. Yet, molds grow best in an atmosphere in which the air is not free moving.

Molds, like yeast, prefer a slightly acid medium. In fact, mold growth has the effect of decreasing the *p*H of a loaf of bread at the point of growth. Nevertheless, both alkaline and acid foods may mold. The first statement leads to the erroneous belief that, when acids or acid salts are added to inhibit "rope," mold growth is stimulated. Ordinary bread is already slightly acid and a little added acidity will have no significant effect on mold growth.

Hoffman, Schweitzer, and Dalby[51] have investigated the fungistatic properties of normal saturated fatty acids containing from 1 to 14 carbon atoms over a *p*H range from 2 to 8. Their effectiveness on mold varies according to the chain length, the concentration of the acid, and the *p*H of the media. However, in general, the longer-chain fatty acids are more effective than the shorter chains. At the neutral point, acids containing 8 to 12 carbon atoms are the most effective for inhibiting mold growth. Unsaturation tends to increase the fungistatic effectiveness of an acid while a branched-chain acid, in general, is less effective than the corresponding straight-chain acid.

This work on acidity and specific acids shows that propionic acid (salts of propionic acids are being used) can be used to retard mold growth. Other fatty acids and their salts undoubtedly would be more effective than propionic; however, their effect on the flavor of the product in which they are used excludes the use of many of them.

The discussion in this section shows that almost any bakery product will mold if moisture and temperature factors are favorable. If the products are wrapped, conditions of moisture will probably be favorable. Thus, the time required for mold to make its appearance will depend on all the factors discussed above and the degree of contamination.

2. Effect of Wrapping and Slicing on Mold Growth

The baker's mold problem was greatly increased with the introduction of moistureproof bread wrappers and moistureproof wrappers for other products, because the wrapper prevents the product from drying out, thus making it a favorable medium upon which mold will grow. The wrapping of bakery products maintains at all times a high humidity around the products which is favorable to growth. Since it is not advisable to give up the sanitary protection afforded by wrappers, considerable research has been undertaken on ways and means of inhibiting mold growth.

---

[51] C. Hoffman, T. R. Schweitzer, and G. Dalby, *Food Research*, **4**, 539 (1939).

The slicing of bread has also increased the difficulty of the mold problem because the bread can become infected in the interior by the carrying of spores on the slicing blades for example. The inside being more moist than the crust, mold growth starts sooner than it would on the outside.

3. Sources of Mold Infection

Since all evidence points to the fact that bakery products are sterile with respect to mold when baked, infection must come from sources which contact the finished products, as, for instance, contaminated air, conveyor belts, racks and machines, handling and paper used as wrapping.

Mold spores are always present in air. The better the sanitary conditions of the bakery, however, the less spores will there be in the air. Since mold spores are ever present, the baker can expect his ingredients, machinery, etc., to be more or less contaminated. Unless proper air conditioning (see below) is applied, it is easy to see how mold spores find their way onto racks, conveyor belts, slicing and wrapping machines, wrapping paper, etc., with which the products will come in contact after they are baked.

Prescott, Streider, and McClellan[52] found that wax wrapping paper (undoubtedly the transparent celluloses can be included) is practically sterile on arrival at the bakery. After the products are wrapped (in modern moistureproof papers) and sealed, there is little possibility of infection unless the wrappers are broken.

Even slightly soiled clothing and hands are likely to carry mold spores. Thus, frequent washing of workers' hands and frequent changes of clothing will help to eliminate chances of contamination.

And, in general, the less the product is handled, the less the possibility of contamination.

4. Precautions for Prevention of Mold Infection

Since mold contamination occurs during the cooling of bakery products, the best way of preventing it is to cool the bread in an atmosphere free of mold spores. Complete air conditioning is the best approach to such a condition.

Although the point of mold infection is different than in the case of "rope," many of the precautions listed for "rope" apply to mold, as: sanitation and cleanliness of ingredient storage, fermentation room and proof box, and store conditions; no stale returns; less moisture in products; quality control, and the use of propionates and diacetates.

---

[52] S. C. Prescott, J. W. Streider, and R. N. McClellan, *Baking Tech.*, 1, 142, 188, 230, 282 (1922).

(a) *Air Conditioning*

It is claimed that good air-conditioning systems (that wash and filter the air) will remove 95% of the mold spores in the air; so air conditioning of the cooling and finishing room will effectively reduce contamination by mold. The products can be transferred directly from the oven into this room, and after cooling can be sliced (in case of bread) and wrapped in the same room.

In order to obtain the necessary efficiency from an air-conditioning unit, it must be handled properly, that is, by frequent changes of air filters, if such are used, and prevention of fungi from growing in the wash water used in the system. The latter can be accomplished by using the proper germicide in the wash water. Carswell, Doubly, and Nason[53] have recommended a mixture of *o*- and *p*-benzyl phenols for this purpose. Cambier[54] and Wells[55] have advocated the use of ultraviolet light in air-conditioning systems to remove bacteria from the air.

(b) *Ultraviolet Radiation*

Besides use in the fermentation room and proof box, germidical ultraviolet radiation has been proposed for use through the entire cooling and finishing room. It seems that an installation of this type would be ineffective, for at least during the summer when windows must be kept open for ventilation, it is possible that mold spores may blow in from the outside faster than they are killed by the ultraviolet lamps. Bakers are cautioned to avoid drafts of outside air, because the air coming in from outside may be contaminated with mold spores. This is a difficult precaution to observe, however, unless an air-conditioning system is employed. Owen and others[56] have found that germicidal ultraviolet radiation is effective, when used properly, in reducing the development of moldy bread. The most effective use seems to be close direct radiation (using reflectors) of the bread as it enters the slicer and wrapper, and of the slicer and wrapper, especially the slicer blades. The radiation tends to keep the mold contamination (also "rope") of the slicer blades at a minimum, and thus reduces contamination of the bread. If the radiation is played on the bread long enough before it enters the slicer and wrapper, it will, undoubtedly, also be effective in reducing surface contamination. Various workers have shown that these ultraviolet radiations at close range will kill most mold spores in a maximum time of one minute.

XII. SCORING OF BAKERY PRODUCTS

Most bakers resort to scientific scoring of their products, thereby being aided in securing quality merchandise and maintaining this quality once it is attained. The

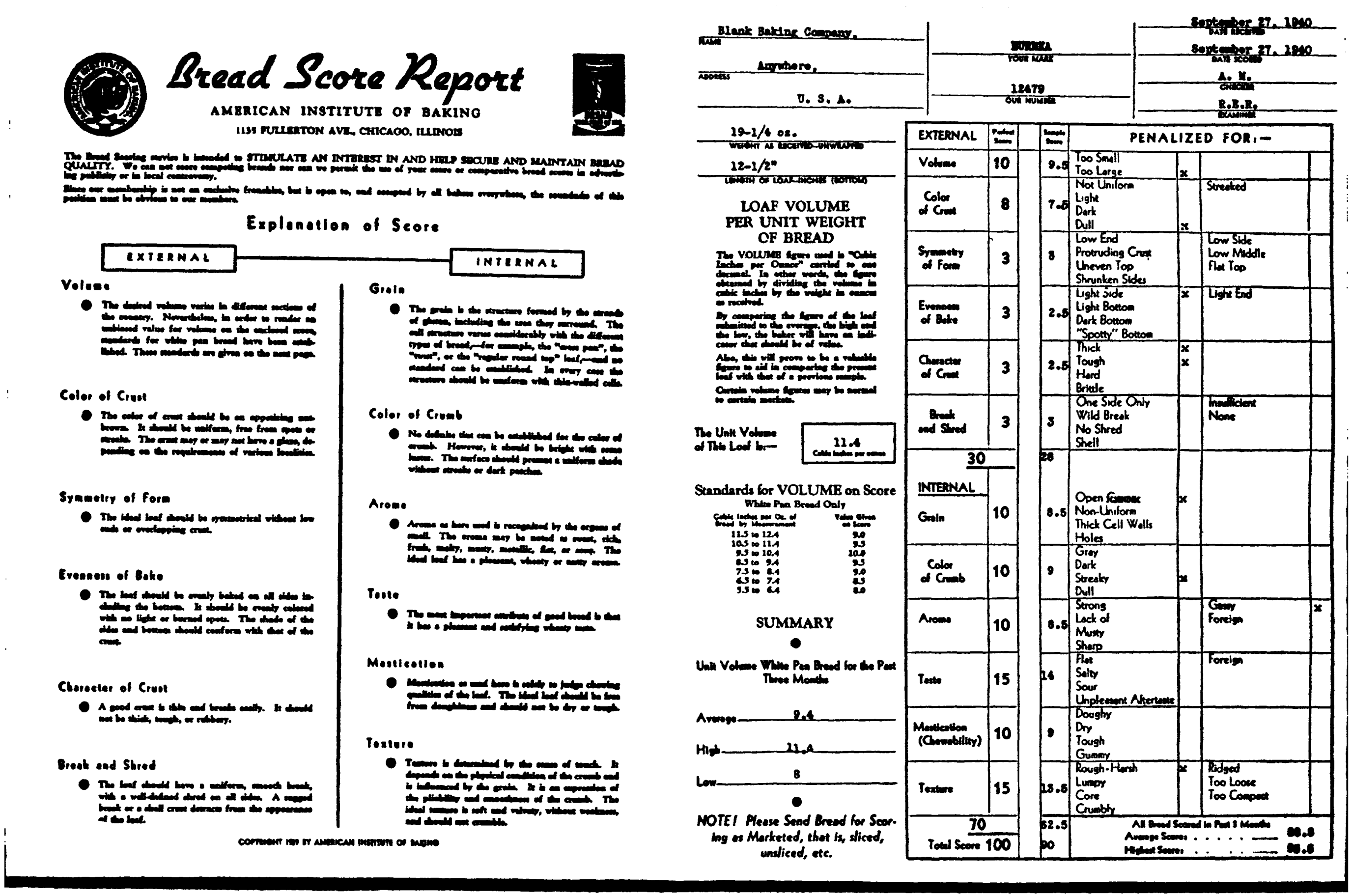
---

[53] T. S. Carswell, J. H. Doubly, and H. K. Nason, *Ind. Eng. Chem.*, **29**, 85 (1937).

[54] M. R. Cambier, *Bull. acad. med.*, **102**, 13 (1929).

[55] W. F. Wells, *J. Ind. Hyg. Toxicol.*, **17**, 253 (1935).

[56] W. L. Owen, *Food Industries*, **4**, 208 (June, 1932).



Bread Score Report

AMERICAN INSTITUTE OF BAKING
1135 FULLERTON AVE., CHICAGO, ILLINOIS

The Bread Scoring service is intended to STIMULATE AN INTEREST IN AND HELP SECURE AND MAINTAIN BREAD QUALITY. We can not score competing brands nor can we permit the use of your score or comparative bread scores in advertising publicity or in local controversy.

Since our membership is not an exclusive franchise, but is open to, and accepted by all bakers everywhere, the soundness of this position must be obvious to our members.

Explanation of Score

EXTERNAL

Volume

- The desired volume varies in different sections of the country. Nevertheless, in order to render an unbiased value for volume on the enclosed score, standards for white pan bread have been established. These standards are given on the next page.

Color of Crust

- The color of crust should be an appetizing nut-brown. It should be uniform, free from spots or streaks. The crust may or may not have a glaze, depending on the requirements of various localities.

Symmetry of Form

- The ideal loaf should be symmetrical without low ends or overlapping crust.

Evenness of Bake

- The loaf should be evenly baked on all sides including the bottom. It should be evenly colored with no light or burned spots. The shade of the sides and bottom should conform with that of the crust.

Character of Crust

- A good crust is thin and breaks easily. It should not be thick, tough, or rubbery.

Break and Shred

- The loaf should have a uniform, smooth break, with a well-defined shred on all sides. A ragged break or a shell crust detracts from the appearance of the loaf.

INTERNAL

Grain

- The grain is the structure formed by the strands of gluten, including the area they surround. The cell structure varies considerably with the different types of bread,—for example, the "oven pan", the "twist", or the "regular round top" loaf,—and no standard can be established. In every case the structure should be uniform with thin-walled cells.

Color of Crumb

- No definite tint can be established for the color of crumb. However, it should be bright with some luster. The surface should present a uniform shade without streaks or dark patches.

Aroma

- Aroma as here used is recognized by the organs of smell. The aroma may be noted as sweet, rich, fresh, malty, musty, metallic, flat, or sour. The ideal loaf has a pleasant, wheaty or nutty aroma.

Taste

- The most important attribute of good bread is that it has a pleasant and satisfying wheaty taste.

Mastication

- Mastication as used here is solely to judge chewing qualities of the loaf. The ideal loaf should be free from doughiness and should not be dry or tough.

Texture

- Texture is determined by the sense of touch. It depends on the physical condition of the crumb and is influenced by the grain. It is an expression of the pliability and smoothness of the crumb. The ideal texture is soft and velvety, without weakness, and should not crumble.

COPYRIGHT 1939 BY AMERICAN INSTITUTE OF BAKING

NAME: Blank Baking Company,
ADDRESS: Anywhere,
U. S. A.

YOUR MARK: EUREKA
OUR NUMBER: 12479

DATE RECEIVED: September 27, 1940
DATE SCORED: September 27, 1940
CHECKER: A. M.
EXAMINER: R.E.R.

WEIGHT AS RECEIVED—UNWRAPPED: 19-1/4 oz.
LENGTH OF LOAF—INCHES (BOTTOM): 12-1/2"

LOAF VOLUME PER UNIT WEIGHT OF BREAD

The VOLUME figure used is "Cubic Inches per Ounce" carried to one decimal. In other words, the figure obtained by dividing the volume in cubic inches by the weight in ounces as received.

By comparing the figure of the loaf submitted to the average, the high and the low, the baker will have an indicator that should be of value.

Also, this will prove to be a valuable figure to aid in comparing the present loaf with that of a previous sample.

Certain volume figures may be normal to certain markets.

The Unit Volume of This Loaf is:— 11.4 Cubic inches per ounce

Standards for VOLUME on Score

White Pan Bread Only

Cubic Inches per Oz. of Bread by Measurement	Value Given on Score
11.5 to 12.4	9.0
10.5 to 11.4	9.5
9.5 to 10.4	10.0
8.5 to 9.4	9.5
7.5 to 8.4	9.0
6.5 to 7.4	8.5
5.5 to 6.4	8.0

SUMMARY

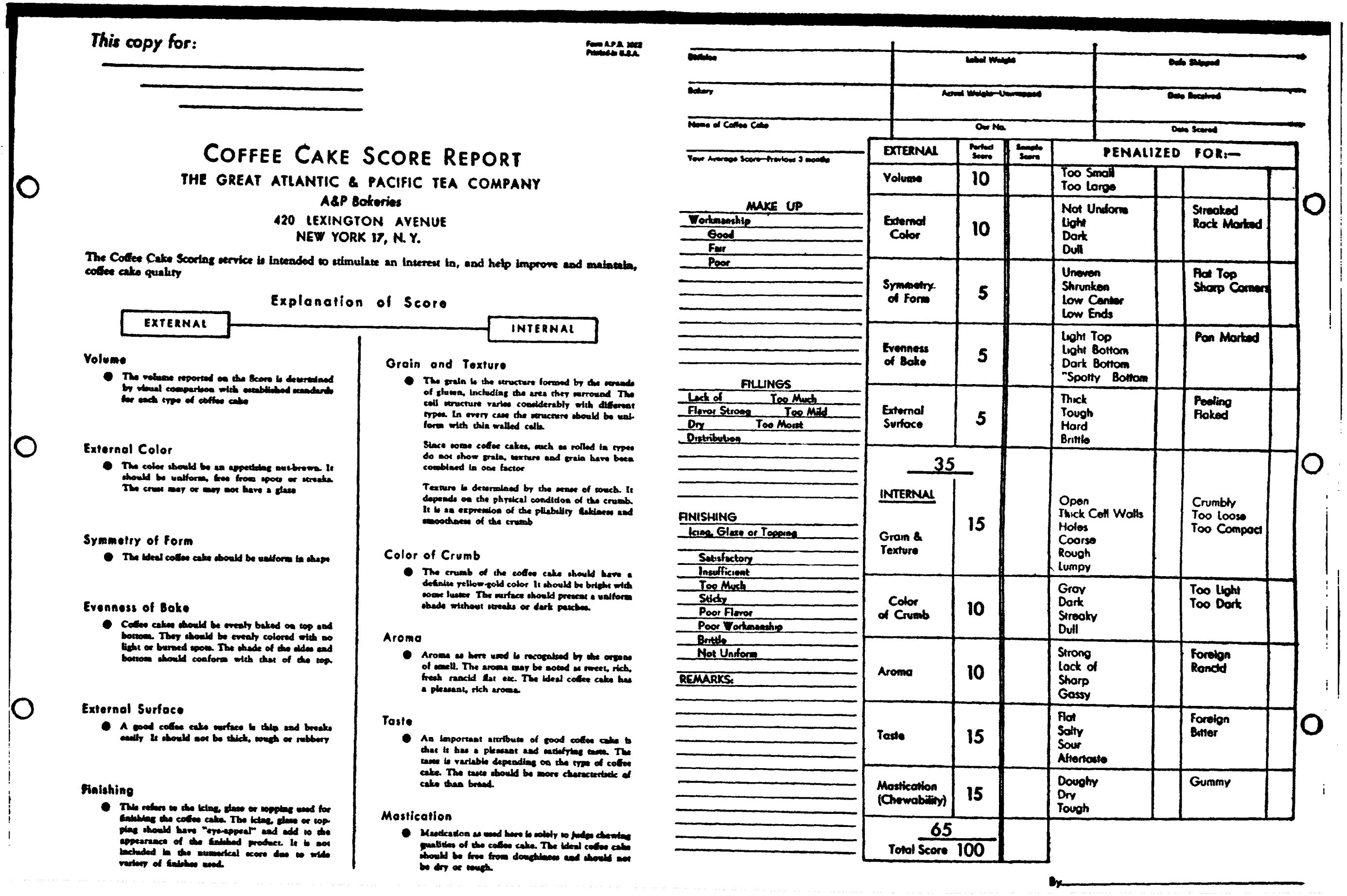
Unit Volume White Pan Bread for the Past Three Months

Average 9.4
High 11.4
Low 8

NOTE! Please Send Bread for Scoring as Marketed, that is, sliced, unsliced, etc.

EXTERNAL	Perfect Score		Sample Score	PENALIZED FOR:—			
Volume	10		9.5	Too Small Too Large	x		
Color of Crust	8		7.5	Not Uniform Light Dark Dull	x	Streaked	
Symmetry of Form	3		3	Low End Protruding Crust Uneven Top Shrunken Sides		Low Side Low Middle Flat Top	
Evenness of Bake	3		2.5	Light Side Light Bottom Dark Bottom "Spotty" Bottom	x	Light End	
Character of Crust	3		2.5	Thick Tough Hard Brittle	x x		
Break and Shred	3		3	One Side Only Wild Break No Shred Shell		Insufficient None	
	30		28				
INTERNAL							
Grain	10		8.5	Open Coarse Non-Uniform Thick Cell Walls Holes	x		
Color of Crumb	10		9	Gray Dark Streaky Dull	x		
Aroma	10		8.5	Strong Lack of Musty Sharp		Gassy Foreign	x
Taste	15		14	Flat Salty Sour Unpleasant Aftertaste		Foreign	
Mastication (Chewability)	10		9	Doughy Dry Tough Gummy			
Texture	15		13.5	Rough-Harsh Lumpy Core Crumbly	x	Ridged Too Loose Too Compact	
	70		62.5	All Bread Scored in Past 3 Months			
Total Score	100		90	Average Score: . . . . . . —— 88.8 Highest Score: . . . . . —— 95.6			

Fig. 64.—Typical Bread Score Report according to the *American Institute of Baking*. At the left is shown the front sheet, which includes an explanation of the score; to the right is the scoring form.



This copy for:

Form A.P.B. 1022
Printed in U.S.A.

COFFEE CAKE SCORE REPORT

THE GREAT ATLANTIC & PACIFIC TEA COMPANY
A&P Bakeries
420 LEXINGTON AVENUE
NEW YORK 17, N. Y.

The Coffee Cake Scoring service is intended to stimulate an interest in, and help improve and maintain, coffee cake quality

Explanation of Score

EXTERNAL

Volume

- The volume reported on the Score is determined by visual comparison with established standards for each type of coffee cake

External Color

- The color should be an appetizing nut-brown. It should be uniform, free from spots or streaks. The crust may or may not have a glaze

Symmetry of Form

- The ideal coffee cake should be uniform in shape

Evenness of Bake

- Coffee cakes should be evenly baked on top and bottom. They should be evenly colored with no light or burned spots. The shade of the sides and bottom should conform with that of the top.

External Surface

- A good coffee cake surface is thin and breaks easily It should not be thick, tough or rubbery

Finishing

- This refers to the icing, glaze or topping used for finishing the coffee cake. The icing, glaze or topping should have "eye-appeal" and add to the appearance of the finished product. It is not included in the numerical score due to wide variety of finishes used.

INTERNAL

Grain and Texture

- The grain is the structure formed by the strands of gluten, including the area they surround The cell structure varies considerably with different types. In every case the structure should be uniform with thin walled cells.

  Since some coffee cakes, such as rolled in types do not show grain, texture and grain have been combined in one factor

  Texture is determined by the sense of touch. It depends on the physical condition of the crumb. It is an expression of the pliability flakiness and smoothness of the crumb

Color of Crumb

- The crumb of the coffee cake should have a definite yellow-gold color It should be bright with some luster The surface should present a uniform shade without streaks or dark patches.

Aroma

- Aroma as here used is recognized by the organs of smell. The aroma may be noted as sweet, rich, fresh rancid flat etc. The ideal coffee cake has a pleasant, rich aroma.

Taste

- An important attribute of good coffee cake is that it has a pleasant and satisfying taste. The taste is variable depending on the type of coffee cake. The taste should be more characteristic of cake than bread.

Mastication

- Mastication as used here is solely to judge chewing qualities of the coffee cake. The ideal coffee cake should be free from doughiness and should not be dry or tough.

Division ______ Label Weight ______ Date Shipped ______
Bakery ______ Actual Weight—Unwrapped ______ Date Received ______
Name of Coffee Cake ______ Our No. ______ Date Scored ______

Your Average Score—Previous 3 months ______

MAKE UP
Workmanship
Good
Fair
Poor

FILLINGS
Lack of ______ Too Much
Flavor Strong ______ Too Mild
Dry ______ Too Moist
Distribution

FINISHING
Icing, Glaze or Topping
Satisfactory
Insufficient
Too Much
Sticky
Poor Flavor
Poor Workmanship
Brittle
Not Uniform

REMARKS:

EXTERNAL	Perfect Score	Sample Score	PENALIZED FOR:—			
Volume	10		Too Small Too Large			
External Color	10		Not Uniform Light Dark Dull		Streaked Rack Marked	
Symmetry of Form	5		Uneven Shrunken Low Center Low Ends		Flat Top Sharp Corners	
Evenness of Bake	5		Light Top Light Bottom Dark Bottom "Spotty Bottom		Pan Marked	
External Surface	5		Thick Tough Hard Brittle		Peeling Flaked	
	35					
INTERNAL						
Grain & Texture	15		Open Thick Cell Walls Holes Coarse Rough Lumpy		Crumbly Too Loose Too Compact	
Color of Crumb	10		Gray Dark Streaky Dull		Too Light Too Dark	
Aroma	10		Strong Lack of Sharp Gassy		Foreign Rancid	
Taste	15		Flat Salty Sour Aftertaste		Foreign Bitter	
Mastication (Chewability)	15		Doughy Dry Tough		Gummy	
	65					
Total Score	100					

By ______

Fig. 65.—Typical Coffee Cake Score Report as used by the A & P Bakeries At the left is shown the front sheet, which includes an explanation of the score; to the right is the scoring form showing the figures which represent perfect scores.

factors controlled are those whose quality is dependent on practical production methods used in the shop.

Figure 64 illustrates a complete bread score. Not only is the bread given a numerical score, but a picture of a slice taken from the center of the loaf is returned to the bakery. The picture shows the baker the internal faults of his loaf. The volume is determined mechanically. The method illustrated is that used by the American Institute of Baking.

Other bakery products are rated in a similar manner. Figure 65 shows samples of the coffee cake scoring method used by the bakeries of the Great Atlantic and Pacific Tea Company. Periodic scoring of all bakery products by the Products Control Laboratory, which has authority to see that corrections are made, is one of the most effective means of maintaining high-quality bakery products.

XIII. PACKAGING

Although packaging is covered elsewhere in these volumes, it is of vital importance to all bakers except the small retail baker. It may therefore be helpful to have a brief outline of packaging as it applies directly to bakery products.

Package design and composition must be such that the package protects the product both in appearance and in freshness. With most bakery products it is a question of not allowing too much moisture to escape. However, with products like cookies and crackers, the main problem is to prevent them from absorbing moisture from the air.

For bread and most cakes, so-called moistureproof wrappers should be used. These are mainly waxed papers and transparent cellulose sheets, which are not really completely moistureproof because they allow some moisture to pass through. However, it is desirable in some localities and with some products to use semimoistureproof cellulose sheets, that is, films allowing more moisture to escape. This is particularly true of sugar doughnuts and all-over iced cakes during the summer months. The amount of moisture that escapes prevents the sugar and the icing, respectively, from becoming damp and sticky.

Cookies and crackers and similar products usually cannot be wrapped in the ordinary waxed or transparent sheets, which are too porous and allow too much moisture to be absorbed by the product from the air, and therefore cause them to become rubbery and lose their crispiness. Cookies are not quite so much a problem as crackers, and in most cases can be wrapped in heavy, moistureproof, transparent cellulose. Special sheets and special means of packaging have had to be developed for crackers, and include, generally, either several wrappings of waxed glassine sheets and/or transparent cellulose sheets. Such products as popcorn and potato chips have even been packaged in airtight cans and in rubberized packages having a very low porosity.

Certain cakes and cookies and doughnuts are packaged in cartons or trays with which they come in contact. These trays must be moistureproof and greaseproof, and must not affect the flavor of the product. Trays fulfilling such requirements have been developed and are being used. They produce more attractive packages which are not soiled by moisture and grease marks.

It should be added that, today, bakers who maintain products control laboratories are in a position to tell the paper and packaging manufacturer what is required in the way of wrapping and packaging material. Such specifications not only make the job of the manufacturer of the packaging material easier, but also result in more suitable packaging for the bakery product.

Chapter XIX

VEGETABLES, MUSHROOMS, NUTS, AND FRUITS

F. A. LEE

Geneva, New York

These groups of food materials, vegetable in origin, are of importance because of their food value, and also because of the flavor and color that they contribute to the pleasure of eating. Some of them are of value mainly because of the relatively large quantities of carbohydrates, fats, or proteins found in them. Others are rich in the food factors known collectively as the vitamins. Still others contribute pungency and are desirable because of it. They all afford the diet a source of minerals and bulk that are necessary for the maintenance of health. The value and importance of these groups of foods cannot be too strongly emphasized.

I. VEGETABLES

The foods that we know as vegetables are derived from many different parts of plants. Some are botanically bulbs, others fruits, roots, shoots, tubers, leaves, stems, and flowers. The larger proportion of our vegetables are, however, fruits, roots, leaves, or stems.

1. Bulbs

(a) Onion

The onion (*Allium cepa*) has been used for centuries. It is a biennial plant with a large single bulb. The bulb is spherical, flattened, or spindle-shaped, and well rounded. The bulb of the multiplier onion is compound. The colors are silvery white, yellow, brownish, and red, and are frequently used as the means of classification. The skin is composed of dried outer scales, and is inedible.

The odor and taste of the onion are due to a volatile oil,[1] allylpropyl disulfide $(C_3H_5)S \cdot S(C_3H_7)$. This compound is volatile, which fact accounts for the milder flavor of cooked onions as compared with those in the raw state. In most cases, white onions are milder than the yellow or red varieties.

Both foreign and domestic types are available. The foreign include Bermuda,

---

[1] F. W. Semmler, *Arch. Pharm.*, **230**, 434 (1892).

Spanish, and Italian onions. These are more tender and mild, but less hardy than the domestic types, and do not keep so well.

Onions are cooked and used as a table vegetable, used raw for salads, and used in flavoring other foods.

Annual total production (average for the years 1929–1938): 14,157,000 100-lb. sacks. Average return to the grower: $1.21 a sack. (From the U. S. Dept. Agr. *Agricultural Statistics*, 1941).

(*b*) *Garlic*

Like the onion, garlic (*Allium sativum*) has been used since prehistoric times. The bulb is made up of several easily separable egg-shaped angular cloves, with a thin, transparent, whitish skin (the scales of the primary bulb) surrounding them.

The active ingredient of the volatile oil[1] is allyl disulfide $(C_3H_5)_2S_2$, allylpropyl disulfide $(C_3H_5)S \cdot S(C_3H_7)$, together with at least one other compound.

Garlic is eaten raw, and is also extensively employed as a flavor in sauces and in cooking.

Annual production (average of the years 1932–1938): 157,000 100-lb. sacks. Value to the grower: $3.32 a sack.

2. Cucurbits

(*a*) *Cucumber*

The cucumber (*Cucumis sativus*) has been cultivated for several thousand years. The fruit is oblong and somewhat triangular in cross section, and is usually more or less smooth when it has reached maturity. The fresh vegetable has a green rind with white inner meat. The salad vegetable is not completely mature. When fully mature, the fruit is yellow or orange in color, but in this condition it is used only in the preparation of certain types of pickles. Very small fruits are used in large quantities for the manufacture of many different kinds of pickles.

Annual production (average for the years 1929–1938): 4,171,000 bu. of 48 lbs. each. Average return to the grower: $0.98 a bushel.

(*b*) *Pumpkin*

The pumpkin (*Cucurbita pepo*) is large, hollow, and nearly spherical, with flattened ends. The rind is colored yellow, orange, or green. The seeds are white, almost elliptical, pointed, and tend to be flat.

The fruit is canned commercially as pie stock, and for this purpose the consistency of the finished product is of prime importance. It has been found that the pumpkin reaches the point at which it yields a pack of highest consistency if it is canned when it is slightly immature or barely ripe. It is for this reason that the commercial canner should start packing the pumpkin as soon as possible if he is to obtain the best pack with the material at hand. Tests indicate that the starch and insoluble solids of the broccoli in the ratio of about 3:2.[4] Small amounts of oxalic and succinic acids were found also. The fresh vegetable contains an average of 1.2 mg. of vitamin C to a gram.

Broccoli is cooked as a table vegetable. It is also canned and quick-frozen to some extent.

(c) *Cauliflower*

Like broccoli, cauliflower (*Brassica oleracea* var. *botrytis*) is a type of cabbage in which the head consists of a white mass of abortive flowers upon modified stems. This mass is surrounded by green leaves.

According to several authors, cellulose, pentosans, methylpentosans, dextrose, and levulose are present. Another found mannite. An average of 0.94 mg. of vitamin C per gram has been found in cauliflower. The vegetable contains 14.3 mg. of iron and 1.4 mg. of copper to the kilo on the fresh basis. The ash is rather high in potash.

Cauliflower is cooked as a table vegetable. It is also frequently employed as an ingredient in mixed pickles. In addition, cauliflower is sometimes quick-frozen.

Annual production (average for the years 1929–1938): 7,284,000 crates containing 37 lbs. each. Average return to the grower: $0.72 a crate.

4. Fruits (except Cucurbits and Legumes)

(a) *Eggplant*

Botanically, eggplant (*Solanum melongena* var. *esculentum*), a rather large, oblong, and purplish-colored fruit, is a berry.

Analyses have shown that the soluble and insoluble solids of the eggplant increase largely in the early stages of growth. The starch, sugars, nitrogen, and solids remain more or less fixed during the late stages of development. Fresh eggplant contains an average amount of 0.07 mg. of vitamin C per gram.[5] The vegetable contains 6.1 mg. of iron and 1 mg. of copper in a kilo of fresh material.

Eggplant is cooked as a table vegetable.

Annual production (average for the years 1929–1938): 822,000 bu. of 33 lbs. each. Average return to the grower: $0.73 a bushel.

(b) *Garden Pepper*

The fruit of the garden pepper (*Capsicum annuum*), like that of the eggplant, is botanically a berry. It is used when it is green or red in color. All varieties of peppers are pungent in varying degrees. This flavor is usually caused by the nitrogenous compound capsaicin, but analyses show little if any of this substance in the peppers used in Serbia.

---

[4] E. K. Nelson and H. H. Mottern, *J. Am. Chem. Soc.*, **53**, 1909 (1931).

[5] D. K. Tressler, N. Y. State Agr. Expt. Sta., *Circ.* **196** (1942).

Red peppers were found to contain 6.0 mg. of iron to the kilo of fresh material. Green peppers showed 4.1 mg. of iron and 1.0 mg. of copper to the kilo on the fresh basis.

Peppers are cooked as a vegetable, and are used raw as an ingredient of salads. They are also used as an ingredient of sauces, and in foods in which the particular flavor is desired.

(*c*) *Okra* (*Gumbo*)

Okra (*Hibiscus esculentus*) is a pod comprising five or more cells with a corresponding number of ribs, containing kidney-shaped seeds. Okra contains 0.15 mg. of vitamin C in 1 g. of fresh material. It is mainly of importance for use in soups partly because of the mucilaginous material it contains, and is grown largely in the South. The immature pods are cooked and eaten as a vegetable, in addition to their use in soups.

(*d*) *Sweet Corn*

Corn (*Zea mays* var. *saccharata*) is found on the market as a long spike covered with the kernels and the whole enclosed in a leafy husk. Silk is seen at the distal end. The types with white or yellow grains are the ones most frequently seen, and at the present time the yellow Golden Cross variety is especially prized for commercial canning and freezing. Sweet corn is most desired as a vegetable when it is in the milk stage. This milkiness is caused by the presence of starch grains suspended in the liquid.

Total sugar increases up to the fifteenth day and then rapidly decreases at first but more slowly in the later maturing stages.[6] Sucrose is low at first, but increases rapidly up to the fifteenth day and then slowly decreases. At the 20-day stage, corn samples have been found to be quite tender and sweet, and in prime canning condition. At the 25-day stage the corn was tougher and less sweet and the flavor not so good as that at the 20-day stage. At the end of 30 days, the corn was tough, of poor flavor, and too mature for canning. It was generally agreed that the 20-day corn yielded the best canned product. In supplementing the information of the authors mentioned, it is well to note that in the northern part of the country these periods are usually increased several days, depending upon the weather. It might also be well to add that prime freezing corn is usually about 3 days younger than that used for canning.

Starch continues to increase in amount as the corn gets older. After the ears have been removed from the stalk, respiration continues rapidly, and sugars are changed to starch.[7] The depletion of sugar in green sweet corn after it has been separated from the stalk does not proceed at a uniform rate but becomes slower and slower, until finally the loss of sugar ceases when the initial total sugar has decreased about 62% and the sucrose about 70%. Calculated on the basis of original moisture, the corn contained, when the depletion of sugar ceased, approximately 1.5% of total sugar as invert sugar, 0.7% of sucrose, and 0.8% of free-reducing substances.

---

[6] C. W. Culpepper and C. A. Magoon, *J. Agr. Research*, **28**, 403 (1924).

[7] C. O. Appleman and J. M. Arthur, *J. Agr. Research*, **17**, 137 (1919).

Reversibility of the chief processes involved in the sugar depletion, resulting in an equilibrium between the rate of sugar loss and the rate of sugar formation, would account for the cessation of actual sugar loss.

Raising the temperature simply hastens the attainment of the equilibrium positions which seem to be about the same for all temperatures. At 30° C., most of the total sugar loss occurs during the first 24 hrs. After storage at 20° C., 25%, and at 10° C. or good refrigerator temperature, only 15%, is depleted during the same period. In general, it may be stated that the rate of sugar loss, until it reaches 60% of the sucrose, is doubled for every increase of 10° C. up to 30° C.

TABLE 154

CHANGES IN THE CHEMICAL COMPOSITION OF CORN DURING THE PROCESS OF RIPENING[a]

Age after silking, days	Moisture, %	Reducing sugars as invert, %	Nonreducing sugars as invert, %	Total polysaccharides as starch, %
Golden Bantam				
5	89.45	3.36	0.65	1.43
10	88.62	3.11	1.64	1.79
15	79.14	1.92	3.97	8.44
20	69.67	0.89	3.41	17.46
25	62.98	0.52	2.30	23.02
30	58.37	0.67	1.47	27.73
Country Gentleman				
5	90.53	3.07	0.74	1.38
10	88.30	2.73	1.64	1.82
15	78.60	1.49	3.82	9.12
20	71.14	1.06	2.89	16.82
25	66.69	0.75	2.27	21.76
30	61.34	0.61	2.07	24.97

[a] C. W. Culpepper and C. A. Magoon, *J. Agr. Research,* **28,** 403 (1924).

The authors further state that respiration alone cannot account for these changes, but that respiration may become an important factor in accelerating depletion of sugar by raising the temperature on the inside of large piles of green corn. Most of the decreases in percentage of sugar in green sweet corn during storage is due to polymerization to polysaccharides, chiefly starch. These facts are illustrated by Tables 155 and 156.

Sweet corn was found to contain 5.1 mg. of iron per kilo of fresh material. Evergreen corn was found to have 1.1 mg. of copper, and Golden Bantam 0.6 mg. of copper per kilo of the fresh sample.

Fresh sweet corn contains from 0.05 to 0.14 mg. of vitamin C to the gram. Canned

TABLE 155

SUGAR LOSS FROM GREEN SWEET CORN (STOWELL'S EVERGREEN) DURING DIFFERENT PERIODS OF STORAGE AT DIFFERENT TEMPERATURES, EXPRESSED AS PERCENTAGE OF THE SAME INITIAL SUGAR AT ALL TEMPERATURES[a]

Hours in storage	Total sugars, %, at storage temperature				
	0° C.	10° C.	20° C.	30° C.	40° C.
24	8.12	16.98	25.61	50.28	45.79
48	14.51	27.95	45.73	57.09	60.15
72	18.03	38.71	55.50	59.00	62.16
96	22.00	49.22	62.10	61.84	. . .

Hours in storage	Sucrose, %, at storage temperature				
	0° C.	10° C.	20° C.	30° C.	40° C.
24	3.51	20.78	31.05	59.42	51.03
48	10.39	37.49	56.12	66.76	64.68
72	15.08	47.46	64.22	68.55	69.24
96	21.25	60.54	70.16	70.19	. . .

TABLE 156

DEPLETION OF SUGAR IN GREEN CORN (STOWELL'S EVERGREEN) BALANCED CHIEFLY BY FORMATION OF POLYSACCHARIDES HYDROLYZED BY DILUTE ACID[a]

Storage Temp., 0° C.

Hours	Total sugars plus polysaccharides as glucose							
	First period		Second period		Third period		Fourth period	
	0	24	24	48	48	72	72	96
Av., %	10.86	10.86	13.62	13.87	11.07	11.29	13.49	13.12

Storage Temp., 10° C.

Hours	First period		Second period		Third period		Fourth period	
	0	24	24	48	48	72	72	96
Av., %	13.18	12.95	14.64	14.70	13.89	13.47	12.55	12.77

Storage Temp., 20° C.

Hours	First period		Second period		Third period		Fourth period	
	0	24	24	48	48	72	72	96
Av., %	11.16	10.53	13.65	13.30	11.35	11.00	12.14	12.11

Storage Temp., 30° C.

Hours	First period		Second period		Third period		Fourth period	
	0	24	24	48	48	72	72	96
Av., %	13.48	12.31	14.43	14.26	12.08	12.78	12.02	12.48

[a] C. O. Appleman and J. M. Arthur, *J. Agr. Research*, **17**, 137 (1919).

corn shows from 0.03 to 0.10 mg., and frozen corn shows 0.07 to 0.11 mg. Fresh sweet corn contains 25.0 I. U. of vitamin A per gram. Frozen corn was shown to contain 26.0 I. U. of this vitamin per gram. These were not corresponding samples. Both, however, were Golden Bantam. Golden Bantam corn contains from 1.2 γ of thiamine (vitamin B_1) to a maximum of 2.0 γ of this substance per gram of fresh sample.[8]

Annual production of corn for processing (average for the years 1929–1938): 676,100 tons. Average return to the grower: $10.24 a ton.

(*e*) *Tomato*

Botanically the tomato (*Lycopersicum esculentum*) is a berry. Most garden tomatoes are irregularly spherical in shape and somewhat flattened at each end. Some Italian tomato paste varieties are elongated and sometimes pear-shaped and plum-shaped. Most tomatoes are red when ripe, but yellow varieties are produced.

In California, Saywell and Cruess[9] found that, in general, the higher the summer temperature, the lower the solids content of the tomatoes. Strangely enough, it was found also that tomatoes picked in November in California were lower in total solids than those picked during September and October.

Practically all the sugar found in tomatoes is the reducing type. Sucrose is present in very limited amounts, and starch probably not at all in the ripe fruit, although some authors assert that minute quantities are present.

It has been found that cold-pressed tomato juice undergoes a lowering in viscosity and consequently a lowering in quality, because of rapid enzymic demethoxylation of the pectin.[10]

It is known that respiration decreases as the fruit increases in size. When maximum size is reached, the respiration again increases until the fruit attains its color, and then it decreases again.[11]

Haber[12] showed that tomatoes stored at 50° F. kept well or, if green, ripened normally. They kept well for several weeks after removal from this storage. Unless quick ripening of green fruit is desired, 70° F. is too high a temperature for more than 5 days.

The coloring matters present are carotene and lycopene. The acid of tomatoes is predominantly citric, and secondarily malic. The relative proportion of the two is debated. In analyzing tomato samples, the total acid is reported as anhydrous citric acid.

Tomatoes are used in enormous quantities, cooked, raw, and in salads. Large numbers are canned each year, and also many are used for the preparation of such products as catsup and chili sauce, as an ingredient in hot sauces, for tomato paste, for tomato juice, and in the green condition as an ingredient of piccalilli.

---

[8] U. S. Dept. Agr., *Circ.* 638 (1942).

[9] L. G. Saywell and W. V. Cruess, Calif. Agr. Expt. Sta., *Bull.* 545 (1932).

[10] Z. I. Kertesz, *Food Research*, **3**, 481 (1938).

[11] F. G. Gustafson, *Plant Physiol.*, **4**, 349 (1929).

[12] E. S. Haber, *Iowa State Coll. J. Sci.*, **5**, 171 (1930–1931).

Fresh tomatoes vary in vitamin C content from 0.1 mg. to 0.45 mg. per gram. Canned tomatoes vary from 0.1 mg. to 0.26 mg. per gram for this same vitamin. Canned tomato juice shows practically the same range as canned tomatoes. Canned tomatoes were found to contain 0.7 to 1.3 γ of vitamin B_1 to the gram. Fresh tomatoes contain 6 mg. of iron and 0.6 mg. of copper per kilo.

Annual production of tomatoes (average for the years 1929–1938): 19,584,000 bu. of about 53 lbs. each. Average return to the grower: $1.26 a bushel. For processing: 1,533,200 tons. Average return to the grower: $12.54 per ton.

5. Leaves and Stems

(*a*) *Brussels Sprouts*

The sprouts or small heads of Brussels sprouts (*Brassica oleracea* var. *gemmifera*) are formed from the buds in the axils of the leaves. These heads resemble cabbage in structure.

Fresh Brussels sprouts have been found to contain 0.7 mg. to 1.8 mg. per gram of vitamin C. Canned, they have been shown to contain 0.5 mg. per gram of sample. The fresh sample of this vegetable was found to contain 1.8 γ of vitamin B_1 to the gram.

Fresh Brussels sprouts contain 22.3 mg. of iron and 1.0 mg. of copper per kilo.

They are cooked and served as a hot vegetable, are canned, and quick-frozen.

(*b*) *Cabbage*

Cabbage (*Brassica oleracea* var. *capitata*) is made up of many rather thick and overlapping leaves attached to a short, thick stem, to form a head. These heads are spherical, pointed, or flattened, according to the variety. Savoy cabbage is characterized by curly leaves and rather excellent flavor. Both white and red varieties are grown.

After the very early stages of development, cabbage shows no really significant changes in chemical composition. Furthermore, important changes evidently do not take place during storage.

The ether-soluble materials of the cabbage leaf are as follows:[13]

Pigments:	
Chlorophyll (*a* and *b*)	9.3%
Carotene	0.5%
Xanthophyll	0.8%
Substances containing phosphorus:	
Calcium phosphatidate	18.4%
Unidentified calcium salts	5.0%
Unidentified iron compound	3.0%
Glycerides and Waxes:	
Containing palmitic, stearic, linoleic, and linolenic acids	17.5%
Glycerol	1.3%

---

[13] A. C. Chibnall and H. J. Channon, *Biochem. J.*, 23, 176 (1929).

Unsaponifiable matter:	
Chiefly nonacosane and di-*n*-tetradecyl ketone	12.3%
Unsaturated fraction:	
Sterols	4.5%
Unidentified products	13.3%

Cabbage contains 3.4 mg. of iron and 0.5 mg. of copper per kilo of fresh material. This vegetable contains 0.2 to 1.8 mg. of vitamin C to a gram of the fresh material. Dried cabbage contains 0.1 to 0.9 mg. of vitamin C in a gram of material.

Cabbage is cooked as a table vegetable, is used raw as an ingredient of salads, is used in slaw, in the preparation of sauerkraut, and in soups.

Annual production (average for the years 1929–1938): 1,134,400 tons. Average return to the grower: $13.87 a ton.

(*c*) *Celery*

The fleshy stalks are the edible part of the celery plant (*Apium graveolens*), although a variety known as celeriac develops a rather large rootstock that is edible.

When celery is held in cold storage at 32° F. sucrose shows a marked increase in the early part of the storage period, eventually declining, with a simultaneous increase in glucose, which in turn reaches a maximum and then declines.[14] (See Table 157).

TABLE 157

CHANGES IN REDUCING SUGARS AND SUCROSE IN CELERY DURING COLD STORAGE AT 32° F.

Days in storage	Reducing sugars, g. % of dry wt.	Sucrose, g. % of dry wt.	Total sugars as glucose, g. % of dry wt.
0	4.7	4.6	9.5
18	5.2	7.6	13.2
47	7.3	4.6	12.1
68	8.0	3.3	11.5
75	8.4	3.5	12.1
103	6.3	3.1	9.6
131	2.2	0.2	2.4

[a] R. H. White-Stevens, *Sci. Agr.*, **17**, 128 (1936).

The ash of celery is rather high in potash, soda, calcium, and phosphates. Celery contains 7.7 mg. of iron and 0.1 mg. of copper per kilo on the fresh weight basis. The vegetable contains 0.05 to 0.18 mg. of vitamin C in a gram of fresh material.

Celery is cooked and used as a hot table vegetable. It also is eaten raw, and is used as an ingredient of soups and salads.

Annual production (average for the years 1929–1938): 9,525,000 crates weighing 90 lbs. each. Average return to the grower: $1.53 a crate.

---

[14] R. H. White-Stevens, *Sci. Agr.*, **17**, 128 (1936).

(d) *Chinese Cabbage*

Heads of Chinese cabbage (*Brassica pe-tsai*) resemble in appearance a cross between a bunch of celery and a head of common cabbage. The vegetable has the length and breadth of celery and the leafiness of cabbage.

It contains 0.04 to 0.27 mg. of vitamin C per gram of the fresh vegetable. On the fresh basis, it contains 5.7 mg. of iron and 0.6 mg. of copper per kilo.

Chinese cabbage has become quite popular in recent years as a salad vegetable. In China, it is usually cooked.

(e) *Endive*

Endive (*Cichorium endiva*) is cultivated as a salad vegetable. Of the several varieties, the green curly type is the one most frequently seen in this country. It is a leafy vegetable, composed mainly of midrib. It is usually slightly bitter in flavor.

Endive contains about 0.13 mg. of vitamin C in a gram of fresh substance. It also contains about 0.6 γ of vitamin B_1.

(f) *Kohlrabi*

Kohlrabi (*Brassica oleracea* var. *caulo-rapa*) is a greatly enlarged stem, resembling the white turnip in flavor but somewhat milder. It is also known as turnip rooted cabbage.

It contains 0.34 to 0.65 mg. of vitamin C per gram of fresh material. Iron is present to the extent of 6.8 mg. and copper to the extent of 1.4 mg. per kilo of the fresh vegetable.

(g) *Lettuce*

Lettuce (*Lactuca sativa*) is a leafy vegetable found on the market in two types—the loose-leaved and the head. As lettuce gets older, the leaves get tougher, and when the plant has reached the seed stage, the leaves are bitter and tough.

Lettuce contains oxalic, *l*-malic, and citric acids in small quantities.[15] Vitamin C is present to the extent of 0.02 to 0.20 mg. per gram of fresh material. Vitamin B_1 is present to the extent of 0.2 to 2.7 γ per gram of fresh material. The leaves contain 18.7 mg. of iron, the head 4.2 mg. of iron, the leaves 0.6 mg. of copper, and the head 0.4 mg. of copper, all to the kilo on the fresh basis.

Lettuce is extensively employed as a salad vegetable.

Annual production (average for the years 1929–1938): 19,536,000 crates of 70 lbs. each. Average return to the grower: $1.49 a crate.

(h) *Parsley*

This dark green, curly-leaved product (*Apium petroselinum*) is employed in soups and salads. It is high in iron, a kilo of the fresh material containing 192 mg. The same quantity of sample contains 2.1 mg. of copper. It contains 1.1 to 2.8 mg. of vitamin C to the gram of fresh material.

---

[15] E. K. Nelson and H. H. Mottern, *J. Am. Chem. Soc.*, **53**, 1909 (1931).

(*i*) *Rhubarb*

The stem of the rhubarb plant (*Rheum rhaponticum*) is the part used as a food. It is long and rather thick, rounded on one side and flat on the other, and usually red or greenish in color.

Culpepper and Caldwell[16] studied the effects of age on the chemical composition of rhubarb. Table 158 illustrates the results obtained.

TABLE 158

COMPOSITION OF RHUBARB PETIOLES AT DIFFERENT STAGES OF DEVELOPMENT. BASED ON PERCENTAGE OF GREEN WEIGHT[a]

Date of sample (1928)	Age, days	Length of petiole, in.	Total solids, %	Solids insoluble in alcohol, %	Total sugar, %	Acids, malic, %	Total N, %
			Series A. Different Harvest Dates				
Apr. 21	10	2–4	5.21	2.25	0.65	1.00	0.211
May 5	24	10–12	4.80	2.36	0.30	0.95	0.203
May 19	38	12–14	5.71	2.91	0.42	0.88	0.178
May 26	45	14–18	5.87	2.97	0.60	1.00	0.155
June 5	55	14–18	5.81	2.85	0.67	0.82	0.127
June 15	65	14–18	6.52	3.56	0.66	0.75	0.130
			Series B. All Harvested One Day				
June 5	5	3–4	5.33	2.49	0.40	1.08	0.198
June 5	8	6–10	4.83	2.23	0.39	1.11	0.160
June 5	11	10–12	4.60	2.12	0.33	1.08	0.136
June 5	15	12–14	4.84	2.12	0.39	1.11	0.142
June 5	19	14–16	4.56	2.00	0.29	1.07	0.149
June 5	25	16–18	5.25	2.28	0.52	1.09	0.127
June 5	32	18–21	5.81	2.65	0.60	1.14	0.134
June 5	40	18–21	6.17	2.80	0.90	1.16	0.130
June 5	55	14–18	5.81	2.85	0.67	0.82	0.127

[a] Adapted from C. W. Culpepper and J. S. Caldwell, *Plant Physiol.*, **7**, 447 (1932).

Considerable attention has been given to the oxalic acid content of rhubarb, but it has been stated that malic acid is the free acid occurring in quantity. Rhubarb contains vitamin C to the extent of 0.09–0.44 mg. to a gram of fresh material. The ash contains considerable potash. Iron has been found to be present in the fresh material to the extent of 8.6 mg. to the kilo, and copper 0.5 mg. to the kilo.

Rhubarb is one of the borderline products from the standpoint of actual use. As an ingredient of pies and sweet sauces, it is prepared for use as a fruit rather than a vegetable.

---

[16] C. W. Culpepper and J. S. Caldwell, *Plant Physiol.*, **7**, 447 (1932).

(j) *Spinach*

Spinach (*Spinacia oleracea*) is an erect, annual herb. The large number of leaves are bunched near the ground, and are smooth.

It has been shown[17] that fall-grown spinach contains more sugar and total solids than that grown in the spring.

It also has been shown[18] that total sugars accumulate at the expense of starch between 29 and 38° F. with the optimum from 29 to 32° F. If, however, the temperature is raised to 45–50° F. the point of equilibrium of the starch-sugar reaction shifts so as to permit much of the sugar to change to starch. At the time of harvest (see Table 159) on October 30, November 13, and November 28, the total sugars for spinach were unusually high, and on those dates a daily mean maximum-minimum as low as 36 and 40° F. was recorded, which shows that the starch-sugar equilibrium mentioned before changes in spinach at about the same temperatures. In the case of spinach the sucrose content increased and the acid-hydrolyzable substances became less as the season advanced in the autumn. This was probably caused by the hydrolysis of the starchlike materials as the temperature lowered.

TABLE 159

SEASONAL CHEMICAL CHANGES IN SPINACH TRIMMED FOR CANNING. DRY WEIGHT BASIS[a]

Date of harvest	Moisture, %	Reducing substances, %	Total sugar, %	Acid-hydrolyzable substances, %	Crude fiber, %	N, %	Alcohol-soluble materials, %
Fall crop (1928)							
Oct. 9	89.70	2.21	6.80	11.5	3.93	5.24	26.06
Oct. 15	89.12	2 41	4.81	11.0	4.32	4.46	30.61
Oct. 22	88.08	2 10	7.33	11.1	3.07	4.66	30.93
Oct. 30	86.49	2 59	17 21	10.0	2.84	4.56	36.58
Nov. 6	86.78	3 12	9 64	11.0	3.27	3.50	34.44
Nov. 13	86.11	2.20	18.40	11.0	2.33	4.47	51.68
Nov. 20	85 57	3 11	11 25	9.1	3.72	4 61	38.74
Nov. 27	82.05	1.92	19 51	9.1	2.76	4.99	44.44
Spring crop (1929)							
May 8	90.70	1.49	5.38	13.0	3.99	4.89	31.88
May 10	89 12	2.90	8 55	13.5	3.54	3.40	34.54
May 14	90 15	1.47	4 82	12.2	. .	4.77	. . .
May 21	90.28	2 81	6.40	12.0	5.67	4.55	31.63
Graded leaves harvested May 21							
Young	89.81	3 55	6.62	11.7	4.33	7.41	32.03
Mature	88 90	2.81	7.43	12.7	3.94	5.09	42.79
Old	86.90	1.90	8.08	13.5	3.43	4.23	52.88

[a] Adapted from F. W. Geise, Maryland Agr. Expt. Sta., *Bull.* **320** (1930).

---

[17] U. S. Dept. Agr., *Bull.* **1265** (1924).

[18] F. W. Geise, Maryland Agr. Expt. Sta., *Bull.* **320** (1930).

Spinach stored at 26° F. tends to accumulate small amounts of moisture, while that stored at 40° F. loses between 2.49 and 3.28%. When stored at room temperature (60–70° F.) the loss of water amounted to 3.07–6.26%. A marked decrease of total sugars occurred when spinach was stored at 40° and at 60–70° F. The greatest loss was noted at the highest temperature. In view of the fact that crude fiber increases under these same conditions, it is obvious that the quality of spinach decreases during storage. The increase in crude fiber in spinach probably parallels a toughening of the vegetable which, in turn, makes it less desirable.

Chibnall isolated from the cytoplasm of spinach leaves a protein called spinacin forming one-fifth of the total protein matter of the cytoplasm. It is soluble in a slight excess of acid or alkali, and is insoluble in water and salt solution. Spinacin contains arginine 6.95%, lysine 8.19%, and histidine 2.34%. Jodidi found that about 50% of the nitrogen of normal healthy spinach is protein in nature. Other authors set the figure a little higher. Spinach protein was found to contain 4.3% of tryptophane.

The ester distillation method for the detection of organic acids showed that oxalic and citric acids were present, as well as small amounts of malic acid. Spinach was found to contain β-carotene, together with traces of the α-form. Considerable amounts of potash and silica are present in the ash. The plant contains 66 mg. of iron and 1.2 mg. of copper per kilo on the fresh basis. Fresh spinach contains 0.13 to 1.18 mg. of vitamin C to the gram. Canned spinach contains 0.05 to 0.47 mg. of vitamin C to the gram of sample. Frozen spinach contains 0.20 to 0.86 mg. of this vitamin to the gram. The vitamin A value for fresh spinach amounts to 66 to 250 I. U. to the gram.

Spinach is canned in enormous quantities. Large amounts are also quick-frozen. The fresh vegetable is cooked and served as a table vegetable.

Annual market production (average for the years 1929–1938): 12,603,000 bu. weighing about 18 lbs. each. Average return to the grower: $0.43 a bushel. Production for processing during the same period: 48,790 tons. Average price paid to the grower: $13.50 a ton.

(*k*) *Swiss Chard*

Swiss chard (*Beta vulgaris* var. *cicla*) is a leafy vegetable of the beet group. Through cultivation, it has developed leaves rather than the root. Chard contains 40.2 mg. of iron and 1.1 mg. of copper per kilo on the fresh basis. Swiss chard contains 0.40 mg. of vitamin C to the gram of fresh material.

6. Legumes

(*a*) *Bean*

Both shell beans and string (snap) beans (*Phaseolus vulgaris*) are grown. The former are obtained by allowing the fruit to dry, and the seeds (beans) are then removed from the shells. Of these, the white and the red kidney are the most popular.

The snap beans are the green or yellow (wax) immature pods. These pods are long and slender, but meaty, and have the immature seeds inside.

Osborne[19] estimated the following percentages of protein in the bean seed: Phaseolin, 15, phaselin 2, alkali-soluble protein, 3.5, alkali-insoluble protein, 3; a total of 23.5%. Other authors report β-phaseolin (phaseolin) and α-phaseolin, this latter in small quantity.

TABLE 160

PRODUCTS OF HYDROLYSIS OF PHASEOLIN[a]

Glycine	0.55%	Phenylalanine	3.25%
Valine	1.04%	Proline	2.77%
Alanine	1.80%	Tryptophane	present
Serine	0.38%	Histidine	3.32%
Leucine	9.65%	Lysine	7.88%
Cystine	0.84%	Arginine	6.11%
Aspartic acid	5.24%	Ammonia	2.06%
Glutamic acid	14.54%		
Tyrosine	2.18%	Total	61.61%

[a] Adapted from T. B. Osborne and S. H. Clapp, *Am. J. Physiol.*, **18**, 295 (1907); A. J. Finks and C. O. Johns, *J. Biol. Chem.*, **41**, 375 (1920).

The carbohydrates of the navy bean are as follows: sugars 1.59, dextrins 3.71, starch 35.20, pentosans 8.37, galactans 1.33, hemicellulose 0.83, and cellulose 3.11%.

Bean seeds have considerable amounts of oxidizing enzymes called dehydrogenases. The ash of the bean contains considerable amounts of potash and phosphates. String beans contain 9.3 mg. of iron on the fresh basis; dried kidney beans 69.2 mg., air-dried basis; dried navy beans 95.2 mg., air-dried basis—based on one kilo of sample for each of these three cases. String beans contain 1.0 mg. of copper per kilo, fresh basis; dried kidney beans 6.5 mg. of copper to the kilo, air-dried basis; and navy beans (dried) 6.9 mg. of copper to the kilo on the air-dried basis.

Snap beans contain 0.10 to 0.28 mg. of vitamin C per gram, fresh basis. Green beans contain about 2.2 γ of carotene per gram on the fresh basis. Recent studies[20] have shown that during the frenching, scalding, and packing for quick-freezing, the beans lose 0.2 γ of carotene per gram, determined on the fresh basis. This difference is not significant.

The green and yellow fresh beans are used as a table vegetable, and large quantities are preserved by canning and quick-freezing. Shell beans are very popular as baked beans and are canned as such. Many other preparations containing beans are in constant use. Quick-frozen baked beans are now obtainable.

Snap Beans.—Annual market production of snap beans (average for the years 1929–1938): 12,076,000 bu. of 30 lbs. each. Average return to the grower: $1.11 a bushel. Production for processing during this same period: 81,500 tons. Average return to the grower: $47.61 a ton.

Dry Beans.—Production for the year 1939: 14,388,000 bags of 100 lbs. each. Avarage return to the grower: $3.24 a bag.

---

[19] T. B. Osborne, *J. Am. Chem. Soc.*, **16**, 633, 703, 757 (1894).

[20] W. I. Zimmerman, D. K. Tressler, and L. A. Maynard, *Food Research*, **5**, 93 (1940).

(*b*) *Lima Beans*

Fordhook Limas

The Lima bean (*Phaseolus lunatus*) is the flat-seeded type also known as Henderson's Bush. The pods are broad and flat and have flattened beans in them. Fordhooks (*Phaseolus limensis*) come in a similar type of pod, but are somewhat larger, and the bean itself is more fleshy.

The following nitrogenous compounds were found to be present in the Lima bean: α-globulin, β-globulin, and an albumin. Its total proteins are deficient in cystine.

TABLE 161

BASIC AMINO ACIDS IN LIMA BEANS[a]

Amino acid	α-Globulin, %	β-Globulin, %	Albumin, %
Cystine	1.60	0.84	1.07
Histidine	3.71	2.62	2.54
Arginine	5.67	5.07	5.74
Lysine	7.84	8.53	5.97
Tryptophane	present	present	present

[a] D. B. Jones, C. E. F. Gersdorff, C. O. Johns, and A. J. Finks, *J. Biol. Chem.*, **53,** 231 (1922).

Lima beans contain 116.6 mg. of iron per kilo on the air-dried basis. The fresh beans contain 21.0 mg. to the kilo. They contain 8.6 mg. of copper per kilo on the air-dried basis.

Green Lima beans contain 0.14 to 0.28 mg. of vitamin C per gram. These were frozen samples. Fresh samples were found to have as high as 0.41 mg. vitamin C per gram of sample. It has been found that about one-third of the vitamin C of Lima beans is lost during blanching in preparation for freezing. It was suggested[21] that a shorter blanching time would remedy this situation, and still inactivate the enzymes. Mature Lima beans were found to contain 4.2 γ of vitamin B_1 in a gram of sample. Fresh Lima beans were found to contain 2.4 γ of carotene to the gram. No significant losses of carotene resulted during the blanching and packing processes in commercial quick-freezing.

Lima beans are cooked as a table vegetable, and are canned and frozen in large quantities for the same purpose. They are also an ingredient of succotash. The flavor of the Fordhook bean is considered superior by many. Dried Lima beans are used in cooking and baking.

Annual market production of lima beans (average for the years 1929–1938): 714,000 bu. of 32 lbs. each. Average return to the grower: $1.39 a bushel. Production for processing during the same period: 16,460 tons. Average return to the grower: $67.39 a ton.

---

[21] D. K. Tressler, G. L. Mack, and R. R. Jenkins, *Food Research*, **2,** 175 (1937).

(*c*) *Soybean*

The soybean (*Soja max*) is a native of Eastern Asia, and has long been cultivated. Soybeans grow in short, hairy pods, usually containing two or three seeds. The seed itself is evenly rounded, rather than the shape characteristic of most other beans. Soybeans of many different colors are known, depending upon the variety.

Soybean flour is made from the ground hulled beans. Soy meal is the product obtained by grinding the cake after the extraction of the oil.

They contain as the main protein, a globulin, glycinin, another globulin, probably phaseolin, and two albumins, legumelin and soylegumelin. The legumelin is present in much larger amounts than the soylegumelin.

TABLE 162

DISTRIBUTION OF NITROGEN IN SOYBEANS[a]

Humin N	2.87%	Total basic N	28.94%
Cystine N	1.46%	Monoamino acid N	48.28%
Histidine N	5.60%	Proline, tryptophane, etc.	2.43%
Arginine N	15.70%	Ammonia N	9.38%
Lysine N	6.18%	Nonprotein N sol. in cold 1% CCl_3-CO_2H in filtrate from colloidal Fe	5.55%

[a] P. S. Hamilton *et al.*, *J. Am. Chem. Soc.*, 45, 815 (1923).

The oil is used for technical purposes, but when it is refined it is edible. It is composed of glycerides of the following fatty acids, all in small amounts, except as noted: lignoceric acid, arachidic acid, stearic acid, palmitic acid (6.8%), oleic acid (33.4%), linoleic acid (51.5%), and linolenic acid. Unsaponifiable matter amounts to 0.6%. This latter contains two phytosterols in addition to other compounds.

The carbohydrates of soybeans are as follows: galactan, pentosans, invert sugar, sucrose, raffinose, starch, cellulose, hemicelluloses, and dextrin. Waxes and organic acids are present also.

Other compounds have been found, such as: lecithin, a saponin of low toxicity, urease, and uricase. Soybean ash is high in potash and phosphates. Iron is present to the extent of 74 mg. to the kilo, dry basis, and copper to the extent of 12 mg. to the kilo, dry basis. They contain 0.30 to 4.05 I. U. of vitamin A per gram, dry basis (6 to 9% moisture).

Soybeans are used extensively as a food for human beings and cattle as well. The soy cake, as well as the oil, is becoming of increasing importance industrially. The flour is of value as a diabetic food because of the relative absence of starch in a number of the varieties. Edible fresh soybeans are highly prized by some as a table vegetable.

The production of soybeans (for the year 1939): 91,272,000 bu. Average return to the growers; $0.81 for a bushel

(*d*) *Lentil*

This seed (*Lens esculenta*) is lens-shaped and very dark in color. Lentils are a good food, but are considerably more popular in Europe than in this country. The ash of the lentil is high in potash and phosphates.

(*e*) *Pea*

The pea (*Pisum sativum*) is found on the market as two types: the smooth-skinned, or field pea type, and the wrinkled, known as sweet or garden peas. The fresh peas are spherical in shape, green in color, and grow in a rather large pod from which they are easily removed. They are about five-sixteenths of an inch in diameter, but this is subject to considerable variation.

It is known that the specific gravity of peas increases as the peas get older.[22] Young and tender peas have a considerably lower specific gravity than the older ones. It is for this reason that brine flotation is so successful for quality separation in the commercial packing of peas. As peas get older, sugars tend to decrease, and the starches increase. Another objective method for the determination of maturity of peas is the estimation of the percentage of alcohol-insoluble solids, which increase with the age of the peas. It has been determined in the laboratory that after picking, peas undergo a loss of sucrose, and simultaneously, an increase in alcohol-insoluble solids.

TABLE 163

CARBOHYDRATE CONTENTS OF DIFFERENT SIZES AND STAGES OF RIPENESS[a]

Dry Weight Basis

Carbohydrate	Small, %	Medium, %	Full grown, green, %	Full grown, ripe, %
Sugars	6.70	6.20	5.90	4.10
Starch	27.30	32.40	32.90	43.40
Cellulose	11.30	10.50	9.60	7.40

[a] C. F. Muttelet, *Compt. rend.*, **180**, 317 (1925).

Jones and Bisson[23] show that after peas are picked, the loss of both sucrose and starch is rapid at 25° C. but is nearly prevented by storing at 0° C. When kept in the pods, reducing sugars stayed constant at 0 to 5° C. but decreased at higher temperatures. Sucrose was lost most rapidly at 35° C.

It was shown, also, that peas, blanched and cooled, kept very well for several days when stored at 30° F. The blanching checked loss of sugar that normally takes place during cold storage.

The proteins of the pea have been found[24] to be (*a*) legumin, a globulin, and the important protein; (*b*) vicilin, a globulin (these two form about 10% of the seed); (*c*) legumelin; (*d*) protoproteose; (*e*) deuteroproteose. A small amount of citric acid has been detected in the pea.

---

[22] F. A. Lee, *Ind. Eng. Chem., Anal. Ed.*, **14**, 241 (1941).

[23] H. A. Jones and C. S. Bisson, *Plant Physiol.*, **7**, 273 (1932).

[24] T. B. Osborne and G. F. Campbell, *J. Am. Chem. Soc.*, **18**, 583 (1896); **20**, 348, 410 (1898).

The statement has been made that green peas are ready for harvest when the absolute weight of sucrose has reached the peak. This is somewhat later than when the percentage is highest.

The ash of the pea is high in potash and phosphates. Green peas contain 17.7 mg. of iron per kilo on the fresh basis, and 2.4 mg. of copper per kilo, also on the fresh basis. Split peas contain 14.0 mg. of copper per kilo, fresh basis.

TABLE 164

HYDROLYSIS OF PEA PROTEINS[a]

Product	Legumin, %	Vicilin, %	Legumelin, %
Glycine	0 38	0.00	0.50
Alanine	2 08	0.50	0.92
Valine	...	0.15	0.69
Leucine	8 00	9.38	9.63
Tyrosine	1 55	2.38	1.56
Cystine		. .	...
Aspartic acid	5 30	5 30	4.11
Glutamic acid	16.97	21 34	12.96
Serine	0 53	.	...
Phenylalanine	3 75	3 82	4.79
Proline	3 22	4.06	3.96
Tryptophane	present	present	present
Arginine	11.71	8.91	5.45
Lysine	4 98	5 40	3.03
Histidine	1 69	2 17	2.27
Ammonia	2 05	2 03	1.26

[a] T. B. Osborne and F. W. Heyl, *J. Biol. Chem.*, 5, 197 (1909).

Peas contain 0.13 to 0.44 mg. of vitamin C per gram of fresh weight green sample. Canned peas contain from 0.03 to 0.24 mg. to the gram of sample of vitamin C. In the processing line for freezing, peas were found to lose about 30% of the total vitamin C.[25] Peas were found to contain from 6.0 to 33.0 I. U. of vitamin A per gram of fresh green sample. Early season peas gave a value of 3.11 γ of vitamin B_1 per gram of fresh sample, and 4.33 γ of this same vitamin per gram of fresh vegetable at the end of the season. The loss during processing amounted to about 5–25%.[26]

Peas are consumed in large quantities as a table vegetable, and are canned and frozen for this same purpose. Dried peas are used for the preparation of soups, and sometimes are soaked for canning purposes.

Annual market production (average for the years 1929–1938): 7,690,000 bu. containing about 30 lbs. of unshelled peas. Average return to the grower: $1.25 a bushel. Production for processing during the same period: 204,140 tons. Average return to the grower: $51.53 a ton.

---

[25] R. R. Jenkins, D. K. Tressler, and G. A. Fitzgerald, *Food Research*, 3, 133 (1938).

[26] J. C. Moyer and D. K. Tressler, *Food Research*, 8, 58 (1943).

7. Roots

(*a*) *Beet*

Botanically, the beet (*Beta vulgaris*) is a tap root. It is medium in size, and deep red in color. The red color is caused by an anthocyanin, betanin.

Beets, like many other crops, tend to toughen during storage. The carbohydrates of the root include sucrose, glucose, fructose, and a small amount of raffinose. Special varieties of beets are grown for the manufacture of sugar and are high in sucrose. These are known as sugar beets. They contain about 11% of sucrose.

The root of the beet is rather high in potash. On the fresh weight basis, it contains 23.6 mg. of iron and 1.9 mg. of copper per kilo. The beet contains about 0.06 mg. of vitamin C to the gram. They are cooked as a table vegetable, and are served pickled.

Annual production (average for the years 1929–1938): 1,942,000 bu. of 52 lbs. each. Average return to the grower: $0.50 a bushel. Production for processing during the same period: 43,700 tons. Average return to the grower: $11.75 a ton.

(*b*) *Carrot*

Like the beet, the carrot (*Daucus carota*) also is a tap root. It is either long and tapering or short and more or less cylindrical, ending in a point. The color is orange-red to yellow.

Table 165 illustrates the fact that the changes taking place during growth are rather small, in so far as composition is concerned.

TABLE 165

COMPOSITION OF CARROTS AT VARIOUS STAGES OF DEVELOPMENT[a]

Date	Moisture, %	Albumin, %	Fat, %	N-free extract, %	Fiber, %	Ash, %
June 9	88.40	1.26	0.44	6.71	0.62	2.57
June 19	88.20	1.17	0.72	6.74	1.62	1.55
June 26	87.40	1.18	0.34	8.55	0.99	1.54
July 3	87.70	1.22	0.57	7.64	1.51	1.36
July 10	87.30	0.98	0.46	9.03	0.90	1.33
July 20	87.50	1.03	0.66	8.44	1.11	1.26
Oct. 15	84.30	1.66	0.50	10.74	1.25	1.55
Oct. 25	86.80	1.99	0.62	7.70	1.55	1.34

[a] U. S. Dept. Agr., *Ann. Rept.*, **1883**, p. 239.

Carotene is the coloring matter found in carrots. It is of special interest because of its close relation to vitamin A. Carrots contain from 17.0 to 84.0 I. U. of vitamin A per gram of fresh sample. Analyses of carrots grown in Europe show considerably higher results. They contain about 0.45 γ of vitamin B_1 per gram of fresh material. The vitamin C content amounts to about 0.06 mg. per gram of fresh material.

The ash is high in potash. Copper is present to the extent of 0.8 mg. to the kilo, fresh basis, and iron to the extent of 10.7 mg. to the kilo, fresh basis.

The carrot is used as a table vegetable. It is consumed raw in salads, and is used as an ingredient in vegetable juices, canned mixed vegetables, and in soups. It is canned as such, and with peas as carrots and peas. It can be quick-frozen.

Annual production in 1939: 16,061,000 bushels containing 50 lbs. each. Average return to grower: $0.59 a bushel.

(*c*) *Parsnip*

The parsnip (*Pastinaca sativa*) is a tap root and fleshy hypocotyl. It contains from 0.06 to 0.40 mg. of vitamin C per gram of fresh material. It contains, also, 10.7 mg. of iron per kilo, fresh basis, and 1.2 mg. of copper per kilo, also on the fresh basis.

(*d*) *Radish*

The edible part of the radish (*Raphanus sativus*) is a tap root that varies in size, shape, and color. The shape varies from small and nearly spherical to cylindrical and elongated. The colors of different varieties are: white, pink, red, purple, black, and red with white tips. They contain about 0.25 mg. of vitamin C to the gram on the fresh basis. They contain 13.6 mg. of iron and 1.6 mg. of copper per kilo on the fresh basis. The radish is used as a salad vegetable.

(*e*) *Rutabaga*

This vegetable (*Brassica campestris*) is a fleshy root, with yellow-colored flesh. It contains 10.7 mg. of iron and 1.5 mg. of copper to the kilo of fresh material. It is cooked, and used in large quantities as a table vegetable.

(*f*) *Sweet Potato*

The sweet potato (*Ipomoea batatas*) is usually considered a tuberous root. It has a brown skin, and yellow flesh.

Storage studies[27] were carried out on the carbohydrate changes occurring in sweet potatoes under three sets of conditions. (See Table 166.) Two varieties were kept in a cellar for approximately 10 days at the curing temperature of 27° C. after which a part was removed to cold storage at 7.8° C., dropping to 4° C. The temperature of the cellar was allowed to drop to between 11.7 and 16.7° C. except at the end of the season when it rose to 21.1° C. The authors concluded that during storage the starch is first converted to reducing sugar and this is used for the manufacture of sucrose. Reducing sugars, rather than sucrose, seem to be consumed during respiration.

The composition of a number of varieties of sweet potatoes was investigated by

---

[27] H. Hasselbring and L. A. Hawkins, *J. Agr. Research*, **3**, 331, 543; **5**, 509 (1915).

Culpepper and Magoon.[28] See Table 167. Sweet potatoes are cured by storing at 80–85° F. with ventilation for 10 days to 2 weeks. After drying, the temperature should be gradually reduced to 55.

TABLE 166

CARBOHYDRATE CHANGES IN SWEET POTATOES DURING STORAGE[a]

Big Stem	Big Stem				So. Queen	Southern Queen			
	Water, %	Starch, %	Reducing sugar as dextrose, %	Sucrose, %		Water, %	Starch, %	Reducing sugar as dextrose, %	Sucrose, %
Cellar storage					Cellar storage				
Oct. 20	73.50	19.07	0.90	1.90	Oct. 23	71.69	22.09	0.39	1.19
Nov. 8	72.99	16.94	1.32	3.51	Nov. 10	68.41	19.87	0.77	2.97
Dec. 6	71.89	16.42	1.40	3.94	Dec. 7	67.69	19.30	0.72	3.50
Jan. 4	72.06	16.02	1.28	4.39	Jan. 11	67.51	19.75	0.75	3.53
Feb. 1	72.18	14.11	1.67	6.06	Feb. 3	68.02	19.22	0.60	3.95
Mar. 1	71.97	13.09	1.44	6.96	Feb. 28	68.00	18.99	0.53	4.05
June 1	72.45	14.62	0.87	5.85	June 4	68.15	20.15	0.55	2.80
Cold storage					Cold storage				
Nov. 8	72.99	16.94	1.32	3.51	Nov. 10	68.41	19.87	0.77	2.97
Dec. 8	72.99	13.31	2.02	6.46	Dec. 8	66.77	17.40	0.59	5.93
Dec. 21	70.77	10.80	1.60	7.33	Dec. 22	67.57	16.48	0.65	6.94

[a] H. Hasselbring and L. A. Hawkins, *J. Agr. Research*, **3**, 331 (1915).

TABLE 167

EFFECT OF COOKING AND CURING ON THE COMPOSITION OF SWEET POTATOES[a]

Variety	Total solids, %	Alcohol-insoluble solids, %	Reducing sugars, %	Sucrose, %	Maltose, %	Polysaccharides, %	Dextrin, %
Big Stem Jersey							
When dug							
Raw	32.26	27.74	0.48	2.48	..	21.59	..
Cooked	32.55	18.29	6.45	2.23	8.64	14.13	0.51
Cured and stored							
Raw	29.68	22.30	0.83	5.31	..	17.84	..
Cooked	29.97	14.97	5.27	5.35	5.84	11.97	1.24
Yellow Jersey							
When dug							
Raw	29.17	24.15	0.61	2.53	..	19.42	..
Cooked	30.32	16.46	5.95	2.92	7.24	13.00	0.32
Cured and stored							
Raw	30.48	21.82	0 91	5.88	..	17.55	..
Cooked	29.62	17.70	6.14	5.67	7.16	10.05	1.46

[a] C. W. Culpepper and C. A. Magoon, *J. Agr. Research*, **33**, 627 (1926).

Dextrose, levulose, and sucrose are the sugars present in the sweet potato. A little over 1% of it is phytin, calculated on the dry basis. Among the enzymes are catalase, laccase, peroxidase, and amylase. Over half of the ash of the sweet potato is potash, and relatively large amounts of phosphates are also present. Iron is present to the extent of 9.2 mg. per kilo, fresh basis, and copper to the extent of 1.5 mg. to the kilo, fresh basis.

---

[28] C. W. Culpepper and C. A. Magoon, U. S. Dept. Agr., *Bull.* **1041** (1922); *Bull.* **1265** (1924); *J. Agr. Research*, **33**, 627 (1926); H. C. Thompson, U. S. Dept. Agr., *Farmer's Bull.* **970** (1918).

The sweet potato is used in large quantities baked or boiled as a table vegetable. In some parts of the country it is used as an ingredient of pie. A considerable amount is canned.

Annual production in 1939: 72,679,000 bu. Average return to the grower: 75.6 cents a bushel.

(*g*) *Turnip*

The turnip (*Brassica rapa*) is a swollen and fleshy tap root. The upper portion is really hypocotyl. Turnips average 0.42 mg. of vitamin C per gram of fresh material. They are used as a table vegetable and as an ingredient in soups.

8. Shoots

Asparagus

Asparagus (*Asparagus officinalis*) is a fleshy shoot or spear on which scales (true leaves) are found. It is usually green in color, but blanched types are grown. This blanching is produced by cultural methods, regardless of the variety. It has been shown that about half of the so-called total protein is true protein. Asparagine is found in asparagus shoots.

TABLE 168

COMPOSITION OF THE ASPARAGUS STALK AT DIFFERENT POINTS ALONG THE STALK FROM BASE TO TIP. EXPRESSED AS PERCENTAGE OF THE FRESH WEIGHT[a]

Date of sampling	Total height of stalk, cm.	Length of segment, cm.	Mean height of segment from base, cm.	Total solids, %	Reducing sugars, %	Non-reducing sugars, %	Acid-hydrolyzable polysaccharides, %	Astringency, %	Acid as citric, %
May 4	20.32	2.5	1.27	9.89	3.08	1.08	1.25	0.070	0.163
May 4	20.32	2.5	3.81	9.05	2.98	0.24	0.95	0.074	...
May 4	20.32	2.5	6.35	8.73	2.94	0.86	0.91	0.084	0.185
May 4	20.32	2.5	8.89	8.38	2.60	0.92	0.80	0.084	0.185
May 4	20.32	2.5	11.43	8.99	2.48	0.64	0.86	0.104	0.227
May 4	20.32	2.5	13.97	9.63	2.10	0.80	0.93	0.127	0.234
May 4	20.32	2.5	16.51	11.19	1.57	0.85	1.00	0.151	0.286
May 4	20.32	2.5	19.05	13.12	0.05	0.24	0.97	0.239	0.306

[a] Adapted from C. W. Culpepper and H. H. Moon, *Plant Physiol*, **14**, 677 (1939).

From Table 168 it can be seen that sugars are highest at the base and decrease rapidly to a low value near the tip. Total astringency and total acids (as citric) are lowest at the base and highest at the tip. Other data presented, but not included in this table, show that total nitrogen is lowest in the basal portion of the stalk, but increases rapidly in amount in regions near the tip.

Storage studies[29] on asparagus indicate that progressive deterioration as evidenced by the reduction in sugar, increase in fiber, even at a temperature slightly above freezing, and especially pronounced at higher temperatures, takes place. It is desirable, therefore, that the product reach the consumer in the shortest time possible. The results show that green asparagus should be maintained at temperatures slightly above freezing.

Asparagus contains about 7.0 γ of carotene to the gram of fresh material. It has been shown[30] that the loss of carotene during processing for freezing is insignificant. Asparagus was shown to contain about 1.8 γ of vitamin B_1 per gram of fresh material. Losses in processing for freezing amounted to 16% of this vitamin at the beginning of the season and about 20% at the end. It was also shown[31] that no significant losses of this vitamin resulted from the frozen samples stored at 10° F. and lower. Vitamin C is present in asparagus to the extent of 0.40 mg. per gram of fresh sample. Fresh asparagus contains 7.9 mg. of iron and 1.4 mg. of copper to the kilo.

Asparagus is used in large quantities as a table vegetable, and much is canned and quick-frozen for this purpose. It is used, also, as an ingredient of soup.

Annual production (average for the years 1929–1938): 5,382,000 crates of 24 lbs. each. Average value to the grower: $1.61 for a crate. Production for processing in California during this period 52,850 tons. Average price: $71.90 a ton.

9. Tubers

(a) *Potato*

The potato (*Solanum tuberosum*) is a swelling at the end of an underground stem. It is known in a number of different shapes—mostly rounded or elongated elliptical, usually with brown-colored skin, although a number of colored varieties are known. An example of the pink-skinned type is the Early Rose potato.

Chemical analysis has shown that potatoes from Idaho, Utah, and Eastern Washington are considerably higher in starch content than those from the eastern part of the country. High starch content is usually associated with excellent baking qualities, and those from the areas just listed are, therefore, considered to be good for this purpose.

Much has been said about the relationship of specific gravity to starch content. Some authors believe that the specific gravity follows the starch content closely enough to be of value in determining total starch. Others disagree. Recently, some authors judged the baking quality of potatoes on the basis of specific gravity by making use of a brine flotation test. It is probable that a more accurate relationship could be obtained by first blanching the samples used.

Considerable work has been done on the composition of potatoes at different stages of development. (See Tables 169 and 170.) One lot of immature Rural New Yorker potatoes was dug October 3. Another lot was allowed to mature in the ground and was dug October 30. The respiration rate at 22° C. was determined in both lots immediately after digging. (See Tables 171 and 172.)

---

[29] C. S. Bisson, H. A. Jones, and W. W. Robbins, Calif. Expt. Sta., *Bull.* **410** (1926).

[30] W. I. Zimmerman, D. K. Tressler, and L. A. Maynard, *Food Research*, **6**, 57 (1941).

[31] J. C. Moyer and D. K. Tressler, *Food Research*, **8**, 58 (1943).

TABLE 169

PERCENTAGE COMPOSITION OF POTATOES DUG AT DIFFERENT STAGES OF DEVELOPMENT AND ANALYZED IMMEDIATELY[a]

Date of digging	Moisture, %	Ash, %	Crude fiber, %	Protein, %	Sugar, %	Starch, %
June 17	83 66	0 91	0 34	1 77	1 03	10 57
June 24	82 72	0 91	0 30	1 85	1 03	11 47
July 8	80 80	0 91	0 32	1 82	0 68	13 80
July 15	79 35	1 00	0 31	2 23	0 30	14 98
July 22	80 20	1 02	0 31	2 24	0 19	14 25
Aug 27	81 02	1 03	0 32	2 17	0 26	13 60

TABLE 170

PERCENTAGE COMPOSITION OF POTATOES DUG AT DIFFERENT STAGES OF DEVELOPMENT AND ANALYZED AT THE END OF THE REST PERIOD, OCTOBER 26[a]

Date of digging	Moisture, %	Ash, %	Crude fiber, %	Protein, %	Total sugar, %	Starch, %
June 17	81 26	1 20	0 47	2 00	0 41	10 82
June 24	81 78	1 10	0 43	1 88	0 41	11 68
July 8	80 09	1 05	0 37	1 93	0 31	13 34
July 15	78 33	1 05	0 37	2 18	0 24	14 76
July 22	79 56	1 06	0 35	2 33	0 19	14 23
Aug 27	79 65	1 11	0 33	2 28	0 21	15 05

TABLE 171

RESPIRATION OF MATURE AND IMMATURE RURAL NEW YORKER POTATOES IMMEDIATELY AFTER DIGGING[a]

Condition of potatoes	Mg CO_2 per kilo per hour (av for succeeding 24-hr periods)										
	1st period	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th
Mature	20 4	18 1	20 0	19 4	13 3	11 1	10 7	7 1	6 8	6 3	7 4
Immature	33 1	23 9	20 1	15 4	13 3	20 6	18 0	13 3	11 1	9 8	8 7

[a] C. O. Appleman and E. V. Miller, *J. Agr Research*, **33**, 569 (1926).

Other experiments similar to the ones recorded here have all shown greater respiratory activity in the immature potatoes immediately after digging. This is probably due to the greater permeability of the tender skin to gaseous exchange.

The work is further discussed by mentioning that the ripening and maturing processes in potatoes may continue during storage, so that by the end of the rest period immature potatoes large enough for seed have practically the same percentage composition and respiratory response as potatoes allowed to mature on the vine, if both are stored under the same conditions.

TABLE 172

RESPIRATION OF MATURE AND IMMATURE RURAL NEW YORKER POTATOES AFTER 4 MONTHS' STORAGE AT 36° F.[a]

Condition of potatoes	Mg. CO_2 per kilo per hour (av. for succeeding 24-hr. periods)								
	1st period	2nd	3rd	4th	5th	6th	7th	8th	9th
Mature	19.3	23.0	22.1	20.7	18.8	15.6	14.1	13.0	10.8
Immature	18.6	22.9	23.0	21.2	18.0	15.5	13.3	11.8	10.8

[a] C. O. Appleman and E. V. Miller, *J. Agr. Research*, **33**, 569 (1926).

TABLE 173

SUGAR AND STARCH CONTENT OF SUMMER HARVESTED GREEN MT. POTATOES STORED IN VAULT WITH ONLY SLIGHT VARIATION IN TEMPERATURE[a]

Temperature Never below 50° F.

Date of analysis	Moisture, %	Total sugar, %		Starch, %	
		Fresh wt.	Dry wt.	Fresh wt.	Dry wt.
Sept. 4	82.06	0.307	1.71	13.38	74.56
Sept. 21	81.56	0.303	1.64	13.43	72.85
Oct. 12	81.10	0.278	1.47	14.16	74.90
Nov. 8	80.77	0.229	1.24	14.07	73.15

TABLE 174

RELATION OF SUGAR AND STARCH CONTENT TO CHANGING LOW TEMPERATURE FOR AUTUMN HARVESTED GREEN MT. POTATOES STORED IN VAULT[a]

Date of analysis	Range of storage temp., ° F.	Moisture, %	Total sugar, %		Starch, %	
			Fresh wt.	Dry wt.	Fresh wt.	Dry wt.
Nov. 28	42–38	80.44	1.10	5.41	14.09	74.50
Dec. 20	38–35	80.26	1.77	8.97	14.01	70.96
Jan. 13	35–29	79.45	2.14	10.39	14.38	69.80
Feb. 13	32–29	80.06	4.15	21.35	12.68	66.10

[a] C. O. Appleman, Maryland Expt. Sta., *Bull.* **167** (1912).

It has been stated[32] that the main chemical changes taking place during storage are as follows: respiratory, sugar formation from starch, and starch re-formation from sugar. (See Tables 173, 174, 175, and 176.) It was noted that sugar accumulates in potatoes at the expense of starch when the temperature falls to 38° F. The rate of accumulation is most rapid between 32 and 29° F. Long exposure to these temperatures is necessary before the accumulation is significant. The maximum of 3 to 4% is reached in 4 to 6 weeks.

---

[32] C. O. Appleman, Maryland Expt. Sta., *Bull.* **167** (1912).

Freezing does not begin until the temperature is between 28 and 26° F. If potatoes which have become sweet through long exposure to the low temperature are brought into a room at 70–75° F. for a week, about four-fifths of the accumulated sugar will be converted back into starch; the remaining fifth will be lost through respiration. A longer exposure of 3 to 4 weeks in a cellar at the temperature of 45–50° F. will remove most of the accumulated sugar.

Samples of potatoes were stored[33] at 2, 5, and 7° C. in atmospheres containing 0, 5, and 20% of carbon dioxide and 21% of oxygen, and at 30, 60, and 90 days samples were removed for determinations of the reducing sugar and sucrose contents of the juice. It was shown that the rapid increase in reducing sugars that occurs in potato tubers stored at 2° C. was inhibited for 30 days by 5% of carbon dioxide and for 60 days by 20% carbon dioxide; after 90 days the values for those treated with carbon dioxide were one-half to one-sixth of those of the controls.

TABLE 175

CHANGE IN PERCENTAGE OF SUGAR AND STARCH IN GREEN MT. POTATOES STORED AT CONSTANT TEMPERATURE[a]

Days in storage	Total sugar, % of fresh weight		Starch, % of fresh weight	
	68–70° F.	30–32° F.	68–70° F.	30–32° F.
7	0.54	0.68	...	...
15	0.54	1.55	14.35	12.88
34	0.55	2.87	14.20	11.53
55	0.62	3.29	13.85	11.10

[a] C. O. Appleman, Maryland Expt. Sta., *Bull.* **167** (1912).

TABLE 176

DISAPPEARANCE OF ACCUMULATED SUGAR FROM GREEN MT. POTATOES BY SHORT STORAGE AT ROOM TEMPERATURE[a]

Stage of storage	Moisture, %	Sugar, %		Starch, %	
		Fresh wt.	Dry wt.	Fresh wt.	Dry wt.
When taken from vault	79.45	2.14	10.41	14.38	69.95
After 8 days' storage at 68–70° F.	78.80	0.46	2.17	16.58	78.21

[a] F. E. Denny and N. C. Thornton, *Contrib. Boyce Thompson Inst.*, **12,** 361 (1942).

At 5° C. the reducing sugar increase was prevented by 5% carbon dioxide for 90 days with Irish Cobbler, and for 30 days with Katahdin; with Green Mountain the 5% carbon dioxide values were kept down to about half of those for the controls. A retarding effect of 5% carbon dioxide was also obtained at 7° C.

Treatment with 20% carbon dioxide although exerting a retarding effect over a period of 30 days at 5 and 7° C. then had the reverse effect, so that at the end of 90 days at 5° C. the reducing sugar values were equal to the controls, and at 7° C. were three to five times those receiving no carbon dioxide. Changing the temperature from 2 to 7° C. reversed the effect of 20% carbon dioxide upon the reducing sugar content.

---

[33] F. E. Denny and N. C. Thornton, *Contrib. Boyce Thompson Inst.*, **12,** 361 (1942).

The general effect of carbon dioxide upon the sucrose content was to greatly increase it, about sixfold at 5 and 7° C. At 2° C., treatment with carbon dioxide first retarded sucrose increase as compared with the untreated, and then greatly increased it. Part of this anomalous effect was explained as being due to the fact that the temporary maximum, which is formed in the sucrose curve of the juice from untreated tubers after about 30 days of storage at 2° C., does not occur in the presence of suitable amounts of carbon dioxide.

Respiration converts starch and sugar into water and carbon dioxide. This is going on continually in stored potatoes, and produces heat. The heating of potatoes in large heaps is due to high respiration and poor ventilation. Potatoes lose weight during storage because of evaporated water and respired carbon dioxide. Warm dry conditions favor evaporation. Loss of carbon dioxide is greatest at high temperatures.

Storage of potatoes for culinary purposes requires a moderately dry well-ventilated cellar with a temperature between 38 and 42° F. Others have stated that 40° F. is the best storage temperature for potatoes.

A globulin, tuberin, has been found in the potato and, also, small amounts of a proteose. Tuberin forms a flocculent coagulum on heating to 60–65° C. but coagulation is not complete until the solutions have been heated for some time at 80° C. Solanin is a glucoside that exists in the tubers in minute amounts. Except under very unusual conditions, the quantity of this substance present is insufficient to cause any ill effects.

Potatoes average about 0.15 mg. of vitamin C to the gram, fresh weight basis. Potato ash is high in potash, and relatively so in phosphates. They contain 8.5 mg. of iron and 1.7 mg. of copper per kilo, on the fresh basis.

Compared with other vegetables, potatoes lose little on boiling.

Potatoes are used in enormous quantities as a cooked table vegetable—boiled, baked, and fried. They are canned as new potatoes, as French fried shoestring potatoes, and as potato cheese sticks. They are also often cut in thin slices and fried as potato chips both domestically and commercially. In 1943 and 1944, the dehydration of potatoes was carried on extensively.

Average production in the year 1939: 363,159,000 bu. Average price paid to the grower: 69.3 cents a bushel.

(*b*) *Jerusalem Artichoke*

This vegetable, *Helianthus tuberosus*, is of interest because it contains much inulin, a fructose starch.

II. MUSHROOMS AND OTHER FUNGI

The mushrooms are a group of nonpoisonous, edible fungi. Like other fungi, they do not contain chlorophyll and are saprophytes. The plant in each case is composed

TABLE 177
PROXIMATE COMPOSITION OF VEGETABLES AND MUSHROOMS[a]

Food	Constituents of the edible portion							
	Water, %	Protein, %	Fat, %	Ash, %	Total carbohydrate, %	Fiber, %	Sugars, %	Starch, %
Artichoke, globe	83.7	2.9	0.4	1.1	11.9	3.2	...	..
Asparagus	93.0	2.2	0.2	0.67	3.9	0.7	1.3	0.4
Asparagus, canned	93.9	1.7	0.1	1.3	3.0	0.5	1.6	1.0
Beans, snap, green, or wax, trimmed pods	88.9	2.4	0.2	0.77	7.7	1.4	0.4	2.2
Beans, snap, canned	94.3	1.0	0.1	1.3	3.3	0.6	1.2	..
Beans, dry navy, kidney, etc.	10.5	22.0	1.5	3.9	62.1	3.9	3.6	35.8
Beans, Lima, fresh	66.5	7.5	0.8	1.71	23.5	1.5	...	..
Beans, Lima, canned	80.0	5.0	0.5	1.5	13.0	1.0	...	..
Beets, red	87.6	1.6	0.1	1.11	9.6	0.9	...	..
Beets, canned	85.5	1.5	0.1	1.4	11.5	0.9	...	..
Broccoli	89.9	3.3	0.2	1.1	5.5	1.3	1.9	..
Brussels sprouts	84.9	4.4	0.5	1.28	8.9	1.3	...	..
Cabbage, green, white, red, savoy	92.4	1.4	0.2	0.75	5.3	1.0	3.5	..
Cabbage, Chinese	95.2	1.4	0.1	0.89	2.4	0.6	0.9	0.2
Carrots	88.2	1.2	0.3	1.02	9.3	1.1	7.5	..
Carrots, canned	89.6	1.0	0.3	1.5	7.6	1.0	...	..
Cauliflower	91.7	2.4	0.2	0.85	4.9	0.9	2.6	..
Cauliflower, canned	94.5	1.0	0.2	1.3	3.0	0.6	...	..
Celery,	93.7	1.3	0.2	1.08	3.7	0.7	1.2	..
Chard, leaves only	91.0	2.6	0.4	1.20	4.8	0.8	0.8	0.1
Corn, sweet								
Av.	73.9	3.7	1.2	0.66	20.5	0.8	4.3	14.6
Young	80.3	2.9	0.8	0.56	15.4	0.6	5.2	8.7
Medium	72.4	3.7	1.1	0.8	22.0	0.9	4.0	16.1
Old	65.7	4.5	1.8	1.0	27.0	0.8	3.2	21.6
Corn, canned	76.0	2.5	0.9	1.0	19.6	0.4	...	..
Cucumber	96.1	0.7	0.1	0.44	2.7	0.5	2.6	..
Eggplant	92.7	1.1	0.2	0.54	5.5	0.9	...	..
Endive	93.3	1.6	0.2	0.89	4.0	0.8	...	..
Garlic	74.2	4.4	0.2	1.18	20.0	1.0	...	..
Jerusalem artichoke	79.5	2.2	0.1	1.17	17.0	0.8	...	..
Kohlrabi	90.1	2.1	0.1	1.05	6.7	1.1	2.2	..
Lentils, dry, whole	11.2	24.7	1.0	3.2	59.9	3.3	...	..
Lettuce	94.8	1.2	0.2	0.91	2.9	0.6	1.6	..
Mushrooms	91.1	...	0.3	1.14	..	0.9	...	..
Mushrooms, canned	93.0	...	0.2	1.0	..	...	...	..
Okra	89.8	1.8	0.2	0.84	7.4	1.0	...	..
Onion (av.)	87.5	1.4	0.2	0.58	10.3	0.8	6.7	0.5
Onion, green (young)	87.6	1.0	0.2	0.6	10.6	1.8	3.7	0.5
Parsley	83.9	3.7	1.0	2.4	9.0	1.8	...	..
Parsnips	78.6	1.5	0.5	1.15	18.2	2.2	9.5	2.4
Peas								
Av.	74.3	6.7	0.4	0.92	17.7	2.2	3.2	8.2
Young	81.4	5.4	0.3	0.77	12.1	1.8	3.3	3.9
Medium	75.8	6.5	0.4	0.93	16.4	2.2	3.8	5.2
Old	65.0	8.2	0.4	1.05	25.4	2.5	2.3	12.3
Peas, canned	85.4	3.3	0.2	1.0	10.1	1.3	4.5	..
Peas, dry, split without seed coat	10.0	24.5	1.0	2.8	61.7	1.2	...	..
Peas, dry, whole, entire seed	11.6	23.8	1.4	3.0	60.2	5.4	...	45.1

TABLE 177 (*Continued*)

PROXIMATE COMPOSITION OF VEGETABLES AND MUSHROOMS[a]

Food	Constituents of the edible portion							
	Water, %	Protein, %	Fat, %	Ash, %	Total carbohydrate, %	Fiber, %	Sugars, %	Starch, %
Peppers								
Av.	91.5	1.4	0.4	0.53	6.2	1.6	2.1	4.2
Green	92.4	1.2	0.2	0.5	5.7	1.4	1.7	4.2
Red	89.2	1.3	0.7	0.7	8.1	1.6	3.0	..
Potatoes	77.8	2.0	0.1	0.99	19.1	0.4	0.9	14.7
Potato chips	3.1	6.7	37.1	4.0	49.1	...	...	37.1
Pumpkin	90.5	1.2	0.2	0.82	7.3	1.3	2.5	2.6
Pumpkin, canned	90.2	1.0	0.3	0.6	7.9	1.2	3.9	0.3
Radishes	93.6	1.2	0.1	0.95	4.2	0.7	3.4	..
Rhubarb	94.9	0.5	0.1	0.72	3.8	0.7	0.4	..
Rhubarb, canned water pack	95.6	0.4	0.4	0.6	3.0	0.8	...	1.9
Rutabagas	89.1	1.1	0.1	0.83	8.9	1.3	6.7	..
Soybeans, fresh	67.0	12.5	6.5	1.5	..	1.5	3.0	1.5
Soybeans, dry edible	7.5	34.9	18.1	4.7	..	5.0	8.4	2.1
Spinach	92.7	2.3	0.3	1.53	3.2	0.6	0.3	..
Spinach, canned	91.8	2.3	0.5	1.9	3.5	0.7	0.8	1.7
Squash, winter	88.6	1.5	0.3	0.83	8.8	1.4	3.9	1.0
Squash, canned	90.2	1.0	0.3	0.6	7.9	1.2	3.9	0.3
Squash, summer	95.0	0.6	0.1	0.44	3.9	0.5	1.0	0.2
Sweet potatoes	68.5	1.8	0.7	1.07	27.9	1.0	5.4	20.2
Sweet potatoes, canned	66.7	1.5	0.2	0.9	30.7	0.6	...	..
Tomatoes	94.1	1.0	0.3	0.57	4.0	0.6	3.4	..
Tomatoes, green	94.7	1.2	0.2	0.6	3.3	0.4	1.2	0.4
Tomatoes, canned	94.2	1.0	0.2	0.7	3.9	0.4	...	..
Turnips	90.9	1.1	0.2	0.73	7.1	1.1	4.6	..

NOTE: All values are for the edible portion of the fresh vegetable unless otherwise specified. The figures for the canned products refer to the entire contents of the can, and not to the drained material. Since a column was not provided for acids in the vegetable, the total acid content of fresh tomatoes, as citric, is 0.51%. The total acid content of the canned tomatoes is 0.40%.

[a] Charlotte Chatfield and Georgian Adams, U. S. Dept. Agr., *Circ.* **549** (1940).

of numerous hyphae that permeate the host or the soil and the edible part, that is, the spore-bearing body of the plant.

Of the edible fungi, the most important commercially is the common mushroom (*Agaricus campestris*). It is grown extensively in dark places for the market and for canning purposes as buttons and sliced mushrooms.

Urea is found in mushrooms. It is said to be formed from amino acids, and from ammonium carbonate in the living cells of the mushrooms.

The following enzymes are known to be present in *A. campestris:* amylase, maltase, glycogenase, protease, and catalase.

Vitamin C is present to the extent of 0.07 mg. per gram of fresh material.

Truffles (species of *Tuber*) are very aromatic and are saprophytes that never come to the surface of the ground. The black truffle (*Tuber melanosporum*) is the best, and the one most frequently found in commerce. Truffles are rather high in potash and phosphates.

Other edible fungi that are reasonably well known include: morels, puffballs, coral mushrooms, spiny mushrooms, and tube-bearing mushrooms.

III. NUTS

A. DOMESTIC NUTS

1. Almond

The almond (*Prunus amygdalus*) is known in the hard- and soft-shelled varieties, the latter being the more highly prized. Large quantities are grown in California. The almond is closely related to the peach, but, unlike the latter, the flesh is tough and not edible. Both bitter and sweet almonds are known.

The protein of importance in this nut is amandin, a globulin. About half of the edible part is fixed oil, which, as might be expected, is bland. It contains glycerides of the following fatty acids: palmitic, oleic, and linoleic. About three-quarters of the whole is oleic. The ash of the almond is high in potash.

Almonds are used as a dessert nut, in candies and confections, and for the production of almond paste. This latter preparation (marzipan) is used for confections and in the production of macaroons.

Total production for the year 1939: 19,200 tons. Average return to the grower: $209 a ton.

2. Chestnut

These nuts (several species of the genus *Castanea*) grow in a spiny bur that opens at maturity and yields two or more brown nuts that are broad at the base and pointed at the end. They differ from most others in that they are starchy, rather than oily. Commonly, they are roasted before eating. They are also used as an ingredient in the preparation of a stuffing for poultry. In some parts of the world a flour is made of them, and in others they are used as a food in place of potatoes.

3. Filbert

The climate of the Pacific Northwest, west of the Cascade Mountains, is the most nearly ideal in this country for the production of filberts (several species of *Corylus*). Here they are grown on the commercial scale.

Total production in 1939: 3750 tons. Average return to the grower: $227 a ton.

4. Hickory Group (Hickory Nut and Pecan)

(*a*) *Hickory Nut*

The hickory nut (several species of *Carya*) has a smooth shell, is of about the same dimensions in length as in breadth, is pointed abruptly at one end, and has some longitudinal ridges. The shell is rather hard. Very excellently flavored nuts are obtained from the shagbark hickory (*Carya ovata*). The big shellbarks (*C. laciniosa*) produce a larger nut, but the flavor is generally considered to be somewhat inferior to that of the nut produced by the shagbark. It is unfortunate that the hickory nut has been more or less neglected in recent years.

(*b*) *Pecan*

The pecan (*Carya illinoensis*) is rather long, somewhat cylindrical and pointed at each end. It is smooth and brownish in color. It is a native nut that is now cultivated extensively in the southern States. The meats contain large amounts of oil, made up of glycerides of the following fatty acids: arachidic, stearic, palmitic, myristic, oleic, and linoleic. It also contains a number of carbohydrates, including among others, sucrose, invert sugar, araban, and methylpentosans.

Total production in 1939: 63,639,000 lbs. Average return to the grower: 9.3 cents a pound.

5. Peanut

The peanut (*Arachis hypogaea*) grows in a two-seeded pod, although other numbers of seeds to the pod are produced. It originated in tropical America. Peanuts are cultivated in the South for the seeds. They are used when roasted as nuts, and for the manufacture of peanut butter, peanut flour, and for the making of peanut oil.

Peanut oil is composed of glycerides of the following fatty acids: lignoceric, arachidic, stearic, palmitic, oleic, and linoleic.

Peanut butter is usually made of a mixture of Spanish and Virginia peanuts. A butter made solely of Spanish peanuts is too oily, and one made only of Virginia peanuts is too dry.

Peanut flour made from the finer grades of peanut meal, ground from pressed kernels only, was found to afford a very satisfactory supplement to wheat flour.[34] It contains four times as much protein, eight times as much fat, and nine times as many mineral ingredients as white wheat flour.

In making bread, it is customary to mix it with wheat flour. It can replace up to 15 or 20% of the wheat flour in recipes for quick breads and simple cake without a change in the recipe. Beyond this quantity, there must be an adjustment made in the amount of liquid used. Thirty per cent replacement is considered about the limit for a satisfactory product. The chief objection to peanut flour is that it may become weevily. Peanuts contain 23.1 mg. of iron and 9.6 mg. of copper per kilo.

Total production in 1939: 1,179,505,000 lbs. Average return to the grower: 3.42 cents a pound.

6. Pinenut

Pinenuts (several species of *Pinus*) are small seeds obtained from members of the pine family. They are brown in color and about half an inch long. They are oily and rather pleasingly flavored. They are found in New Mexico and Nevada.

---

[34] U. S. Dept. Agr., *Misc. Pub.* **416** (1941); H. S. Bailey and J. A. LeClerc, U. S. Dept. Agr., *Yearbook*, **1917**, p. 289.

7. Walnut Group

(a) *Black Walnut*

The nut (*Juglans nigra*) is nearly spherical and very rough on the outside. Parts of the outer pericarp usually continue to adhere to it. The shells are hard. The wood from the tree of the Eastern American nut is valuable in furniture making. The nut is not so important as the English walnut, but it is used in confectionery.

(b) *English (Persian) Walnut*

The nut (*Juglans regia*) found on the market today is light brown in color because it is bleached in regular commercial practice. It is somewhat pointed at one end, and the shell can be very easily separated into two halves, exposing the kernel. The shell is easily cracked, and is relatively smooth, compared with the other members of this group.

The kernel of the walnut produces an ash that is very high in phosphates: over 50% of the ash is this material. The kernel is rather high in oil, which is highly desired by some as a salad oil. The globulin present in the English walnut is known, and is called juglansin. The green English walnut is very high in vitamin C. It can be consumed in this state after pickling. This high vitamin C content was noted by Pyke, Melville, and Sarson.[35] The English walnut is widely used as a dessert nut and in connection with other foods.

(c) *Butternut*

The nut (*Juglans cinerea*) is oblong, cylindrical, sharp-pointed at the apex, bluntly rounded at the base, jagged over the surface and thick-shelled. Butternuts are pleasing in certain confections, but have been rather neglected.

B. TROPICAL AND SUBTROPICAL NUTS

1. Brazil Nut

The Brazil nut (*Bertholetia nobilis*) is rather large and triangular in cross section. The outer surface of the shell is dark brown to almost black in color. The tree grows along the rivers of Brazil. These nuts are rich in oil that is made up of glycerides of the following fatty acids: myristic, palmitic, stearic, oleic, and linoleic. Brazil nuts contain 10.2 γ of vitamin B_1 to the gram.

U. S. import in 1938–1939: 8,933,000 lbs. of the shelled nuts; 20,585,000 lbs. of them unshelled.

2. Cashew Nut

The cashew nut (*Anacardium occidentalis*) roughly resembles a letter "c" in shape. It contains irritating components, which, however, are destroyed by heat. The roasted kernel is a very palatable nut. Large quantities are produced in India.

U. S. import in 1938–1939: 29,350,000 lbs.

---

[35] M. Pyke, R. Melville, and H. Sarson, *Nature*, **150**, 267 (1942).

3. Coconut

The coconut (*Cocos nucifera*) is obtained from one of the world's most important trees. Coconuts are 5 to 6 inches in diameter, and often larger, and almost spherical in shape. When the hard brown shell is removed, the white meat covered with a brown skin is exposed. The hollow center is partly filled with coconut milk.

Coconut meat contains about 0.02 mg. of vitamin C per gram, fresh. The coconut is quite high in oil, which is composed of glycerides of the following fatty acids: palmitic, stearic, oleic, caprylic, myristic, and lauric. The carbohydrates present in dried copra (dried coconut meat) include: sucrose, raffinose, galactose, fructose, glucose, pentoses, cellulose, pentosans, starch, dextrin, and galactan. The meat is shredded and sold for cakes and confectionery.

Coconut oil is manufactured from copra. This oil is extensively employed in the manufacture of oleomargarine, shortenings, and soap. Coconuts are produced in the Philippines and the South Pacific area.

U. S. import during 1938–1939: 467,470,000 lbs. of copra.

4. Macadamia

Two types of macadamia (*Macadamia ternifolia* var. *integrifolia*) are known, the smooth-shell, which is the nut of commerce, and the rough-shell. The smooth-shell is relatively smooth-surfaced and ovoid to spherical in shape. The shells are one-sixteenth to one-fourth of an inch in thickness and are very hard to crack.[36] Both types grow in Hawaii. They are marketed mostly in the cooked, salted-kernel form, and are vacuum packed in glass jars.

5. Pistachio Nut

This is a small nut (*Pistacia vera*) the cotyledons of which are green in color and covered by a purplish skin. The flavor is distinctive and excellent. The nut is much used in pastries, cakes, ices, and confectionery. It is frequently sold salted in the shell. It is produced in Persia, Syria, and the Mediterranean region.

U. S. import in 1938–1939: 2,336,000 lbs.

IV. FRUITS

A fruit is the matured ovary, with its seeds, and any parts of the flower that are closely associated with it. The edible part of most fruits consists of the fleshy part of the pericarp. In some cases, the edible substance is highly specialized tissue other than the pericarp. In still other cases, the pericarp is combined with other tissues to form the edible material. While nuts are botanically fruits, they differ from our table fruits in that the seed of the nut is the food substance, rather than the pericarp. Fruits in general are acidic and sweet or sugary in character.

---

[36] R. H. Moltzau and J. C. Ripperton, Hawaii Agr. Expt. Sta., *Bull.* 83 (1939).

1. Berries (except Grapes)

A berry is a small fleshy fruit that is pulpy and succulent throughout, and contains seeds imbedded within this pulpy tissue.

(a) *Strawberry*

The strawberry (*Fragaria chiloensis*) is botanically a receptacle, in reality a stem structure. The real fruits of the strawberry are the numerous achenes seen somewhat imbedded at the surface of the succulent receptacle. Many strawberries are irregularly heart-shaped, others are almost spherical. They are red in color.

Strawberries have shown an evolution of 151 mg. of carbon dioxide to the kilo in an hour at 26.2° C. and 17 mg. at 2°C.[36a] This means much faster respiration at high room temperature than at slightly above freezing, which is what one would expect.

The strawberry has been shown to contain citric acid, with a smaller amount of *l*-malic acid.[37] Strawberries contain 0.29 to 0.88 mg. of vitamin C to the gram, fresh basis. They contain 6.6 mg. of iron and 0.2 mg. of copper per kilo of fresh material. Strawberry ash is high in potash.

Strawberries are a favorite dessert berry, and enormous quantities are quick-frozen sliced or whole, with sugar. Large numbers are consumed fresh. Quite a few are canned, and many are made into jam. Strawberry is a favorite flavor for ices, ice cream, and as a topping for ice cream.

Annual production (average for the years 1929–1938): 11,176,000 crates. Average return to the grower: $2.67 a crate of 36 lbs. each.

(b) *Red Raspberry*

The fruit (*Rubus idaeus*) is an aggregate. Many pistils ripen to yield drupelets that more or less cling together. These drupelets separate from the receptacle when the ripe fruit is picked, resulting in a bowl- or cup-shaped arrangement. The berry is red in color.

Respiration studies showed that at 30.8° C. Cuthbert raspberries gave an evolution of carbon dioxide amounting to 311 mg. for a kilo of sample in an hour. At 2.1° C. the same quantity evolved 22 mg. in an hour.

The acid of the raspberry is largely citric, with a small amount of *l*-malic. The ash of raspberries is high in potash and phosphates. A kilo of the berries contains 9.9

[36a] H. C. Gore, U. S. Dept. Agr., Bur. Chem., *Bull.* **142** (1911).

[37] E. K. Nelson, *J. Am. Chem. Soc.*, **47**, 1177 (1925).

mg. of iron and 1.3 mg. of copper, on the fresh basis. They contain from 0.19 to 0.37 mg. of vitamin C to the gram.

They are consumed fresh in large quantities, and a great many are quick-frozen. Many are canned and made into jelly and jam. Like strawberry products, those of the raspberry are favorites at the soda fountain.

(*c*) *Blackberry*

Like the raspberry, the blackberry (several species of *Rubus*) is an aggregate of drupelets, but these not only cling together, but are firmly attached to the receptacle so that the latter does not separate when the fruit is picked.

They contain optically active isocitric acid, some *l*-malic acid, and traces of oxalic, succinic, and citric acids.

Respiration studies showed that a kilo of the fruit at 30.8° C. gave an evolution of carbon dioxide amounting to 239 mg. in an hour. At 1.2° C. an evolution of 18 mg. of carbon dioxide was noted in an hour by a kilo of fruit.

The blackberry is used as a dessert berry, and is canned and made into jam. Juices are made of all these berries.

(*d*) *Loganberry*

The loganberry is of especial interest because it is a very successful hybrid. It is a cross between a variety of the European raspberry and the California dewberry (*Rubus vitifolius*). It is rather large and dark red in color. As with the blackberry, the drupelets are firmly fastened to the receptacle.

The acids present are mainly citric, with a small amount of *l*-malic. The loganberry contains 0.36 mg. of vitamin C per gram of fresh material.

Large quantities of these berries are produced on the Pacific coast for canning and for the fresh market. The berry cooks well, and the flavor is excellent.

(*e*) *Currant*

The currant (*Ribes rubrum*) is a true berry that grows in clusters, much like grapes. It is a native of Europe. While several varieties of currants are known, the one described here is very important commercially.

Respiration studies showed an evolution of 56 mg. of carbon dioxide for a kilo in an hour at 30.2° C. At 0.8° C. the weight of carbon dioxide given off in an hour by a kilo of material amounted to 5 mg.

The acid of the currant is largely citric, but some authors found small amounts of malic acid also. They contain about 0.30 mg. of vitamin C to the gram of fresh material. The ash of the currant is very high in potash. The fresh fruit contains 7.0 mg. of iron in a kilo.

Currants are extensively used in the preparation of jams, jellies, and preserves. The jelly forms rather easily because of the high-grade pectin contained in the fruit.

Zante currants (*Vitis vinifera*), used in the preparation of fruit cake and similar products, are not true currants but are small dried grapes (see page 772) grown mainly in southern Greece. They are also grown in California and Australia.

(*f*) *Gooseberry*

The gooseberry (*Ribes hirtellum*) is a reddish berry, spherical or nearly so, smooth, and about 1 to 2 cm. in diameter. The European gooseberry is larger than the American, and is hairy or prickly.

Authorities agree that the acids are largely citric, with a small amount of malic. Potash is present in rather large amounts in the ash of gooseberries. The fresh berries contain 4.7 mg. of iron and 0.8 mg. of copper in a kilo.

The berries are used for jam, sauce, and pie, and are commercially canned.

(*g*) *Huckleberry*

The common black huckleberry (*Gaylussacia resinosa*) is the most important species and the most abundant. The fruit is not a true berry botanically, but a berry-like ten-celled drupe. The fruit is used in the fresh state for sauces, pies, and muffins. It is also dried, frozen, and canned.

(*h*) *Blueberry*

The fruit (several species of *Vaccinium*) is botanically a many-seeded berry. It is smooth-surfaced, blue-black in color with bloom, and about 1 cm. in diameter.

The acid of the blueberry was found to be citric, with a small amount of *l*-malic. The meager data available show that blueberries contain about 1 I. U. of vitamin A to a gram of fresh sample. The ash of the blueberry is rather high in potash. A kilo of the fresh berries contains 4.1 mg. of iron and 1.1 mg. of copper.

Like the huckleberry, the blueberry is used in the preparation of sauces, pies, and muffins. It is quick-frozen and canned.

(*i*) *Cranberry*

The cranberry (*Vaccinium macrocarpon*) is a native of the western hemisphere. The berry is red throughout, and is nearly spherical or oblong. It is grown in bogs where the area is cool and moist. The Cape Cod area and New Jersey are famous for this product, but excellent berries are grown elsewhere.

It has been determined that berries left in cold storage lose small amounts of sugar and acid, which losses, without doubt, are caused by respiration.

The acids present are mostly citric, but malic and benzoic acids are also present. The ash of the cranberry is high in potash, and relatively so in lime and phosphates. The berries contain 0.9 mg. of copper to the kilo on the fresh basis. They are used extensively for the preparation of sauce, jelly, and juice.

Total production for 1939: 704,100 bbl. of 100 lbs. each. Average return to the grower: $9.85 a barrel.

2. Citrus Fruits

(a) *Orange*

The fruit (*Citrus sinensis*) is almost spherical in shape, orange in color, smooth, and contains a large amount of pulp held in a varying number of segments loosely joined together and to the skin. The navel orange is seedless and was so named because of the umbilical mark at the apex of the fruit. This is caused by the formation of a second small fruit.

Much work has been done on the changes that take place during the ripening of the fruit and its subsequent storage. Bigelow and Gore[38] showed that storage at room temperature resulted in a slight loss of acid and total sugar but a large increase in reducing sugars and a corresponding decrease in sucrose. Losses were caused by respiration. It was shown that Valencia oranges give off 23 mg. of carbon dioxide for a kilo in an hour at 29.3° C. but only 2.0 mg. for a kilo in an hour at 1.7° C.

TABLE 178

CHANGES IN COMPOSITION OF THE SWEET ORANGE DURING RIPENING[a]

Stage of ripening	Solids, %	Total nitrogen, %	Nitrogen lead tannin ppt., %	Nitrogen lead tannin filtrate, %	Acid as citric, %	Reducing sugars, %	Sucrose, %	Ash, %
Green	9.79	0.1008	0.0518	0.0490	0.10	6.13	0.57	0.42
2nd period	10.14	0.0644	0.0194	0.0450	0.17	6.85	1.25	0.37
3rd period	11.52	0.0650	0.0286	0.0364	0.19	6.85	0.71	0.41
Ripe	10.66	0.0705	0.0259	0.0446	0.14	7.95	1.05	0.36

[a] F. Scurti and G. de Plato, *Staz. sper. agrar. ital.*, **41**, 435 (1908).

The acid in the orange is citric, with some malic. Considerable quantities of pectin can be extracted from the white portions of the orange peel. The glucoside, hesperidin, is found in the green fruit. Inosite has been found in small quantity in the juice of the orange. The following materials were isolated from orange juice: ethyl alcohol, acetone, acetaldehyde, formic acid, olefin alcohol, isoamyl alcohol, phenylethyl alcohol, and esters of formic, acetic, and caprylic acids. Geraniol and terpineol also seemed to be present. The orange contains vitamin C to the extent of 0.27–0.70 mg. to a gram of the fresh sample. The ash of the orange contains considerable amount of potash, and is quite rich in lime. The pulp of the orange contains 6.6 mg. of iron to the kilo, fresh basis, and the juice 2.8 mg. to the kilo on the same basis.

Large numbers of oranges are used as dessert fruits. Many are now pressed for juice that is served fresh, is canned, and is frozen. Marmalade is another product that uses many oranges.

Total production in 1939: 75,646,000 boxes. Average return to the grower: $0.94 a box.

---

[38] W. D. Bigelow and H. C. Gore, *J. Am. Chem. Soc.*, **29**, 767 (1907); F. Scurti and G. de Plato, *Staz. sper. agrar. ital.*, **41**, 435 (1908).

(*b*) *Tangerine*

Both the tangerine and the mandarin orange have the botanical designation *Citrus nobilis* var. *deliciosa*. They are flattened in appearance and have segments that separate from themselves and from the skin very easily. The membrane covering each segment is considerably tougher than that of the sweet orange. β-Carotene has been isolated from the fruit flesh and the rind of the mandarin orange by Zechmeister and Tuzson.[39] Tangerine pulp contains 6.1 mg. of iron to the kilo, fresh basis, and 0.9 mg. of copper to the kilo, fresh basis. Tangerines are eaten as a dessert fruit. They are canned in Japan.

(*c*) *Lemon*

The lemon (*Citrus limonia*) is about $2^1/_2$ to $3^1/_2$ in. long, terminating in a nipple-like end, and is composed of 8 to 10 segments closely adhering to the skin. It is yellow in color, and very acid. It is produced in large quantities in the Mediterranean region, and in California, and to some extent in Florida.

Respiration studies on California lemons showed an evolution of 17 mg. of carbon dioxide at 28.4° C. from a kilo of fruit, and 2 mg. at 1.7° C.

Citric acid, the acid of lemons, is present to the extent of 6 to 8% in the juice. Acidity of lemons has been found to be highest in the early autumn. Lemons have the lowest oil content in late winter and spring and highest in the autumn.[40] Small quantities of inosite have been found in lemon juice.

Lemon rind is an especially good source of commercial pectin. Of the citrus fruits, lemon is perhaps the best source of pectin, followed by the orange, and then the grapefruit.

It has been shown that lemons slightly green, but in condition to be harvested, change to yellow on storage for 30 to 60 days at 10–13° C. and air with relative humidity of 80%. If ethylene is added to the air, the ripening can be materially hastened.[41]

Lemon ash is high in potash, phosphates, and lime. Lemon juice contains 1.5 mg. of iron to the kilo, fresh basis. The pulp contains 0.4 mg. of copper to the kilo on the fresh basis.

Lemons are used for the juice, for the sugared rind, for the preparation of flavoring, and the manufacture of pectin.

Total California production for 1939: 11,963,000 boxes. Average return to the grower: $1.54 per box.

(*d*) *Grapefruit*

Grapefruit (*Citrus grandis*) is largely produced in Florida, Texas, Arizona, California, and the West Indies. It is a large, almost spherical, smooth-skinned fruit, that is yellow in color when ripe. It is about 4 to 6 in. in diameter.

---

[39] L. Zechmeister and P. Tuzson, *Z. physiol. chem.*, **221**, 278 (1933).

[40] E. M. Chace, C. P. Wilson, and C. G. Church, U. S. Dept. Agr., *Bull.* **993** (1921).

[41] F. E. Denny, *J. Agr. Research*, **27**, 757 (1924).

Table 179 illustrates the increase in total acid during warm storage. There is evidence indicating that there might be a slight decrease in sugar during warm storage. Table 180 shows the decrease in acid after 4 months' storage while the quantity of sugar stays relatively constant, at 32° F.

TABLE 179

PERCENTAGES OF ACID, SUGAR, AND DRY MATTER IN THE PULP OF GRAPEFRUIT STORED AT 70° F.[a]

Constituent	First harvest, %		Fourth harvest, %	
	Placed in storage July 29	Sampled Oct. 8	Placed in storage Oct. 30	Sampled Jan. 3
Acid as citric	1.55	1.79	1.01	1.20
Reducing sugars as glucose	1.54	3.00	3.00	4.08
Cane sugar as glucose	2.13	1.23	3.18	2.65
Total solids	11.29	11.27	9.45	10.10

TABLE 180

PERCENTAGES OF ACID, SUGARS, AND DRY MATTER IN PULP OF GRAPEFRUIT STORED AT 32° F. FOR 4 MONTHS[a]

Constituent	First lot, %		Third lot, %	
	When placed in storage	After 4 mo. storage	When placed in storage	After 4 mo. storage
Acid as citric	1.06	0.98	1.15	1.10
Reducing sugars as glucose	2.46	2.61	3.70	3.29
Cane sugar as glucose	2.63	2.65	3.25	3.59
Total solids	9.54	9.46	10.30	9.80

[a] Adapted from L. A. Hawkins, *J. Agr. Research*, **22**, 263 (1921).

As in the other citrus fruits described small amounts of inosite are present in the juice. The ash of grapefruit is high in potash and relatively so in phosphates. The pulp contains 2.7 mg. of iron and 0.3 mg. of copper per kilo on the fresh basis.

It is largely used as a breakfast fruit and for preparation of juice. The rind is a source of pectin. Large quantities of the grapefruit and the juice are canned.

Total production for 1939: 35,175,000 boxes. Average return to the grower: $0.44 per box.

(*e*) *Lime*

Lime (*Citrus aurantifolia*) is a small greenish fruit, thin-skinned, with much pulp that is greenish in color and very acid in taste. It is grown in Florida and the West Indies.

Limes contain from 0.07 to 0.49 mg. of vitamin C to the gram of fresh material. The lime is used almost entirely for its juice.

Total production for the year 1939: 95,000 boxes. Average return to the growers: $2.80 per box.

(*f*) *Citron*

The fruit (*Citrus medica*) is about 8 in. long, is lemon-yellow in color, and has a thick skin. The rind is brined, after which it is candied and becomes the citron of commerce.

3. Drupes

The fruit is a single seed, surrounded by stony and fleshy pericarp. The fleshy pericarp is the edible part.

(*a*) *Apricot*

The apricot (*Prunus armeniaca*) is shaped much like the peach, has a nearly smooth skin and is orange-yellow in color throughout. The stone is somewhat lens-shaped and smooth.

Total acids, present to the extent of about 1%, are made up of *l*-malic and citric acids. Apricots contain 40 I. U. of vitamin A per gram of fresh material. The ash of the apricot is high in potash and phosphates. The dried stoned fruit contains 72.6 mg. of iron and 3.7 mg. of copper to the kilo.

Apricots are grown in large quantities in California, but can be produced in most temperate climates. They are eaten fresh, are canned, dried, and are used for making jams and jellies. Oil is extracted from the seed.

Total production for 1939: 322,700 tons. Average return to the grower: $33.74 a ton. In terms of weight of fresh fruit, 225,500 tons were dried, and 64,387 tons were canned.

(*b*) *Cherry*

The fruit of the cherry (*Prunus avium*) is small, spherical or heart-shaped, soft- or hard-fleshed, and colored light red or very dark red. The latter color is almost black. Cherries are classed in four groups: (*1*) Mazzards. (*2*) Hearts—heart-shaped and soft-fleshed. (*3*) Bigarreaus—heart-shaped and hard-fleshed. The Royal Ann or Napoleon belongs to this group. (*4*) Dukes—the juice is acid and the flesh soft.

TABLE 181

COMPOSITION OF UNRIPE AND RIPE NAPOLEON CHERRIES[a]

Date	Sugars, %	Acids as malic, %	*p*H	Nonsugar solids, %
1926				
June 4	10.6	0.90	3.79	3.9
June 25	18.8	0.71	3.81	7.1
1927				
June 23	7.4	0.75	3.68	3.4
Aug. 1	16.5	0.72	3.96	6.2

[a] After H. Hartman and D. E. Bullis, Oregon Agr. Expt. Sta., *Bull.* 247 (1929).

Aside from losses in weight and volume sweet cherries manifest no striking physiological or chemical changes immediately following removal from the tree. There is definitely no improvement in quality of sweet cherries after picking. In no case was there found an apparent increase in sweetness or amelioration of acidity.

Table 181 illustrates chemical changes taking place during the ripening of the Napoleon (Royal Ann) cherries.

While there is some disagreement, it is probable that the acid of the cherry is malic. The ash of the cherry contains reasonably large amounts of potash and phosphates. Red cherries contain 4.6 mg. of iron and black contain 5.1 mg. of iron per kilo on a fresh basis. Red fruits contain 1.4 mg. of copper to the kilo on a fresh basis.

The sour cherry (*Prunus cerasus*) resembles the sweet in composition. It is too sour for dessert purposes, but is used in large quantities for pie stock. The sweet cherry is used as a dessert fruit, is canned, and dried.

Total production of sweet and sour cherries for 1939: 187,010 tons. Average return to the grower: $59.87 per ton.

(*c*) *Peach*

The peach (*Prunus persica*) is almost spherical in shape, with a groove on one side. It has a hairy skin, and is classified as clingstone or freestone, depending on the adherence or nonadherence of the flesh to the stone, and is further classified as white or yellow, depending upon the color of the flesh. The flesh around the stone is frequently red in color.

TABLE 182

COMPOSITION OF THE FLESH OF THE DEVELOPING PEACH (ELBERTA)[a]

Date, 1939	Moisture, %	Reducing sugars as glucose, %	Sucrose, %	Fat, %	Protein N × 6.25, %	Ash, %
June 11	85.68	4.02	0.50	0.91	2.15	..
June 22	86.30	4.33	0.46	0.68	1.56	0.41
July 8	84.92	4.26	1.35	0.39	1.39	0.38
July 21	84.80	3.81	1.43	0.65	1.33	0.56
Aug. 3	85.85	3.64	1.83	0.52	1.75	0.54
Aug. 19	86.32	4.24	1.86	0.26	1.14	0.61
Sept. 19	88.46	3.53	3.37	0.14	0.82	0.32
Sept. 27	87.05	2.94	6.93	0.15	0.83	0.34

[a] After F. A. Lee and H. B. Tukey, *Botan. Gaz.*, **104**, 348 (1942).

Respiration studies showed an evolution of 134 mg. of carbon dioxide to the kilo for 1 hr. at 31.4° C. and 6 mg. of carbon dioxide at 1.1° C. from 1 kilo of ripe fruit.

Work on the peach has shown that during the process of ripening and softening protopectin changes to pectin. This softening is favored by heat and is inhibited by cold.

Experiments have demonstrated that equal quantities of *l*-malic and citric acids are present in the peach.

Peaches contain 3 to 30 I. U. vitamin A per gram of fresh pulp. Of the total biologically active carotenoid content, one-third to one-half is due to β-carotene.

TABLE 183

CHANGES IN COMPOSITION OF PEACH AND RESISTANCE TO THE PRESSURE TEST AT DIFFERENT STAGES OF RIPENESS (ELBERTA)[a]

Stage of ripeness	No. days before or after shipping ripeness	Solids, %	Acid as citric, %	Reducing sugars, %	Sucrose, %	Astringency, %	Pressure test, g.
Unripe	5–6	13.00	0 67	3.32	4.53	0.27	428
Ripe	0	14 26	0 50	3 59	5 52	0.24	294
Overripe	6–7	14 73	0.36	3.80	6.86	0.21	132

[a] Adapted from C. W. Culpepper and J. S. Caldwell, U. S Dept. Agr., *Tech. Bull.* **196** (1930).

Furthermore, peeling results in about a 25% loss in provitamin A but freezing induces no additional loss.[42] Vitamin C contents vary from 0.02 to 0.15 mg. per gram.

The ash of the peach is about 50% potash. Iron is present to the extent of 3.6 mg. per kilo, fresh basis, and about 60.6 mg. to the kilo in dried peaches as sold. The dried fruit contains 2.7 mg. to the kilo of copper on a fresh basis.

Total production in 1939: 61,072,000 bu. Average return to the grower: $0.82 a bushel. In terms of weight of fresh fruit, 5,833,000 bu. were dried, and 13,912,000 bu. were canned.

The nectarine is a smooth-skinned peach belonging to the same genus and species.

(*d*) *Plum*

The plum (*Prunus domestica*) is shaped much like the apricot or peach, is about the size of the former, but some forms are elongated. It has a smooth skin that ordinarily peels rather easily, and is colored, red, yellow, green, or blue.

Respiration experiments gave 103 mg. of carbon dioxide to the kilo in an hour at 30.7° C. and 6 mg. at 1.7° C. of fresh material.

Malic acid appears to be the chief acid of plums. The ash of the plum contains approximately 70% potash. Fresh plums contain 7.7 mg. of iron to the kilo, dried prunes 51.7 mg. to the kilo as sold; blue plums, 1.5 mg. of copper to the kilo of fresh pulp and dried prunes 4.1 mg. to the kilo as sold. Plums contain relatively small amounts of vitamin C and vitamin B_1.

Fresh prunes and plums are similar fruits, the difference being that prunes include only those yielding a satisfactory dried product which in turn depends on the relatively high sugar content.

Plums are served fresh as dessert fruit, are dried, and canned. Plum jam is very popular.

---

[42] D. DeFelice, *Food Research*, **7**, 16 (1942).

Total plum production for Michigan and California in 1939: 77,300 tons. Average return to the grower: $41.83 a ton. Total prune production for Oregon, Washington, Idaho, and California during that year: 673,600 tons. Average return to the grower: $23.09 a ton. In the same year, 32,500 tons of prunes were canned.

4. Grapes

(a) *American Grape*

The grape grows in bunches and the fruit is a black, red, or greenish-yellow berry. The skin and flesh of the fruit are tough. Concord, black, Catawba, red, and Niagara, white, are well-known varieties of *Vitis labrusca* in the eastern and the eastern north central parts of the United States.

TABLE 184

COMPOSITION OF CONCORD GRAPES BEFORE AND AFTER RIPENING (GROWN AT SANDUSKY, OHIO, 1912)[a]

Date	Condition of fruit	Total solids, %	Total sugar as invert, %	Total acid as tartaric, %	Crude ash, %
Sept. 4	Nearly all berries colored	12.69	8.60	1.91	0.28
Sept. 17	Fruit ripening	14.90	12.10	1.14	0.27
Oct. 1	Apparently ripe, quality low	17.77	14.80	1.02	0.41
Oct. 16	Excellent condition, wine ripe	19.34	15.92	0.95	0.39
Oct. 23	Sound, excellent condition	19.31	15.87	0.93	0.41
Nov. 8	Frost about ruined fruit	19.05	15.61	0.90	0.45

[a] Considerable data for a large number of American grapes, grown under different conditions and in different places, have been given by W. B. Alwood *et al.*, U. S. Dept. Agr., *Bull.* **335** (1916).

It was demonstrated that about 60% of the acid of the Concord grape is *l*-malic and the remaining 40% is *d*-tartaric.[43] Some American grapes contain sucrose though it has been stated that European grapes contain only invert sugar. Anderson[44] isolated the pigment of Concord grape and found it to be the monoglucoside of the monoethyl ester of delphinidin. About 50% of the ash of the American grape is potash. For Concord grapes, the skin contains 13.6 mg. and the pulp 7.4 mg. of iron to the kilo on a fresh basis.

Production for North Atlantic states in 1939: 105,480 tons of grapes. Average return to the grower: $36.32 a ton.

(b) *European Grape*

The European grape (*Vitis vinifera*) has a more elongated berry than the American grape; furthermore, the skin is more tender and usually edible, and the pulp is tender. In California, the Muscat of Alexandria, Tokay, Thompson Seedless, Malaga, and other varieties are grown. Zante currants are small, dried grapes grown mainly in southern Greece.

---

[43] E. K. Nelson, *J. Am. Chem. Soc.*, **47**, 1177 (1925).

[44] R. J. Anderson, *J. Biol. Chem.*, **57**, 795 (1923).

Respiration studies on the Flame Tokay grape demonstrated that 57 mg. of carbon dioxide to the kilo in an hour were formed at 34.2° C., and 5 mg. to the kilo at 1.8° C.

During ripening sugar increases and acid decreases. Both malic and tartaric are present, but during the process of ripening tartaric acid remains constant while the malic acid content decreases. The skin of dark European grapes contains oenin, a monoglucoside of dimethyldelphinidin as demonstrated by Willstätter and Zollinger.[45] Comparatively small amounts of vitamin C are present in these grapes. The ash contains about 50% potash and lesser amounts of phosphates. Malaga grapes contain 22.8 mg. of iron to the kilo on the fresh basis. Seeded raisins contain 69.9 mg., seedless raisins 41.3 mg., and dried Zante currants 47.4 mg. per kilo as sold. Malaga grapes contain 0.9 mg. copper to the kilo on the fresh basis, seeded raisins, 2.7 mg., and seedless raisins 2.0 mg. as marketed. Dried currants contain 11.2 mg. of copper per kilo.

TABLE 185

COMPOSITION OF MUSCAT GRAPE JUICE DURING GROWTH AND RIPENING[a]

Date (1917)	Total solids, %	Sugar, %	Total acid as tartaric, %	Nitrogen as protein, %
June 12	5.35	0.91	2.93	0.38
Aug. 7	16.58	12.72	1.60	0.55
Sept. 5	29.36	23.28	0.60	0.65
Sept. 26	32.89	26.68	0.56	0.59

[a] Adapted from F. T. Biolleti, W. V. Cruess, and H. Davi, *Univ. Calif. Pub. Agr. Sci.*, 3, 103 (1918).

Large quantities of grapes are sold fresh for dessert purposes. Many more are used for the manufacture of wines and brandy, and enormous quantities of Muscat of Alexandria and Thompson Seedless for the manufacture of raisins. Thompson Seedless grape is used also as an ingredient of canned fruit cocktail, and some Muscat grapes are canned as such.

5. Melons

(*a*) *Muskmelon*

The muskmelon (*Cucumis melo*) is almost spherical or somewhat elongated with narrow grooves or ribs formed longitudinally on the surface. It is found on the market in a number of varieties.

Immature melons, as may be expected, have much less sucrose than ripe ones. Melons picked before ripeness is reached will lose some sugar in storage because of respiration. The invert sugar changes to sucrose, a change hastened by the presence of ethylene. The seeds of the melons at market ripeness contain less than 0.5% starch, which can be used as an index of the ripeness of melons.

They contain from 0.1 to 0.4 mg. vitamin C to the gram, 5.1 mg. iron to the kilo, and 0.6 mg. of copper, on the fresh basis. These melons are used almost entirely as breakfast and dessert fruit.

---

[45] R. Willstätter and E. H. Zollinger, *Ann.*, 408, 83 (1915).

Total production in 1939: 14,402,000 crates of 60 lbs. each. Average return to the grower: $0.98 per crate.

(b) *Watermelon*

The watermelon (*Citrullus vulgaris*) is large, long, smooth-surfaced, solid green or striped. The edible part is solid throughout, and is usually red or pink, sweet and juicy, and contains many seeds. Yellow-fleshed watermelons are known.

Both reducing sugars and sucrose are known to be present in the ripe fruit. It is rather low in vitamin C, containing 0.01 to 0.1 mg. to the gram of fresh material.

Annual production (average for the period 1929 to 1938): 68,900,000 melons. Average return to the grower: $112 for a thousand melons.

6. Pomes

The pome is a fruit formed by the enlargement of the receptacle which becomes fleshy and surrounds the carpels.

(a) *Apple*

The apple (*Pyrus malus*) is more or less spherical, with a depression at each end. The skin is smooth, sometimes russeted, tightly adhering to the flesh, and varies in color from green through yellow to red. The flesh is white or yellowish, occasionally reddish. The core, which runs through the center from depression to depression, consists of the five carpels containing seeds.

It has been found that *l*-malic is the only acid usually occurring in the apple. Some varieties show very small amounts of other acids such as citric. Power and Chesnut[46] found the amyl esters of formic, acetic, and caproic acids with small amounts of caprylic ester and considerable amounts of acetaldehyde. The waxy material of the peel is known to consist of three substances, triacontane (m. p. 63.5–64), heptacosanol (m. p. 81–81.5), and malol (m. p. 284–285° C).[47]

It is known that peroxidase, an organic peroxide, and tannin are responsible for the darkening of apple tissue.

Respiration studies indicated 65 mg. of carbon dioxide were liberated per kilo in an hour at 34.1° C. and 3 mg. at 2.5° C.

In the early stage of ripening the pectic substances of apples are present mostly in the form of protopectin. During ripening on the tree this constituent decreases while pectin increases and reaches a maximum when the fruit is tree-ripe. Afterward all pectic constituents decrease. This latter change takes place in cold storage at a slower rate.

---

[46] F. B. Power and V. K. Chesnut, *J. Am. Chem. Soc.*, **42**, 1509 (1920).

[47] C. E. Sando, *J. Biol. Chem.*, **56**, 457 (1923).

Ripening is correlated with loss of water, acid, starch, dextrin, and substances hydrolyzed by acid, and a gain in specific gravity, sugars, and soluble pectin. Some investigators found that it was better to put apples in cold storage after ripening on the tree, while others found little difference in composition after the fruit had been held for some time regardless of the stage of ripeness when it was harvested.

TABLE 186

COMPOSITION OF BALDWIN APPLE AT DIFFERENT STAGES OF GROWTH[a]

Stage of growth	Water, %	Reducing sugars, %	Sucrose, %	Starch, %	Acid as malic, %	Ash, %
Very green	81.53	6.40	1.63	4.14	1.14	0.27
Green	79.81	6.46	4.05	3.67	..	..
Ripe	80.36	7.70	6.81	0.17	0.65	0.27
Overripe	80.30	8.81	5.28	..	0.48	0.28

[a] C. A. Browne, Jr., Penn. Dept. Agr., *Bull.* **58** (1899).

TABLE 187

CONSECUTIVE ANALYSIS OF NORTHERN SPY APPLES HELD IN COMMON STORAGE[a]

Date	Total solids, %	Acid as malic, %	Starch, %	Reducing sugars as invert, %	Sucrose, %	Total nitrogen, %	Ash, %
Northern Spy Apples in Common Storage							
1902							
Aug. 25	14.60	1.04	2.61	5.06	1.94	0.032	0.285
Sept. 15	15.39	1.00	2.58	5.80	2.19	0.025	...
Oct. 6	16.03	0.71	1.50	6.03	3.02	0.028	0.329
Nov. 7	16.22	0.70	0	6.90	4.90	0.025	...
Dec. 19	15.63	0.42	0	8.88	2.30	...	...
Winesap Apples in Cold Storage, Picked Sept. 15							
1902							
Oct. 20	16.84	0.68	0.86	8.01	3.47		
1903							
Jan. 19	16.65	0 61	0	9.28	2.51		
Feb. 16	17.10	0.61	0	9.51	2.48		
Mar. 31	17.03	0.54	0	9.96	2.53		
May 5	17.63	0.55	0	10.03	2.14		
Dec. 17	16.89	0.44	0	10.14	0.97		
1904							
Apr. 27	16.60	0.32	0	10.60	0.84		

[a] Adapted from W. D. Bigelow, H. C. Gore, and B. J. Howard, U. S. Dept. Agr., Bur. Chem., *Bull.* **94** (1905).

The apple contains 0.02 to 0.15 mg. of vitamin C to the gram of fresh material. Over 50% of the ash of the apple is potash. Considerable quantities of phosphate are present also. It has been found in the past that good keeping qualities are associated with a high content of phosphates and potash. Iron is present to the extent of about 6 mg. to the kilo, on the fresh basis, and copper about 1 mg.

The apple is extensively used as a dessert and cooking fruit. It is used in the preparation of cider and juice, apple jelly, apple butter, canned, dried, and dehydrated apples.

Total commercial production for the year 1939: 143,085,000 bu. Average return to the grower: $0.64 a bushel.

(b) *Pear*

The pear (*Pyrus communis*) is much like the apple in character. The fruit is large at the calyx end and tapers off toward the stem. The skin is smooth, but rough if the fruit is russet. It is usually yellow in color when market-ripe, and sometimes has red cheeks. Brownish pears are also known. Pears are customarily harvested when green and allowed to ripen in storage. For canning and other large quantity purposes, ethylene gas is often used to speed ripening in storage.

When pears are stored, it is known that sugars increase, while starchy materials decrease. Cold storage merely slows these changes. Seckel pears respired 23 mg. of carbon dioxide in 1 hr. from a kilo of sample at 26.3° C. The same weight gave off 3 mg. of carbon dioxide at 0.9° C.

Acetaldehyde occurs in ripe pears. Not a trace of this substance could be found in pears analyzed immediately after removal from the tree, whatever the stage of maturity.[48] After two days in the ripening room at 22–24° C. the tissues contained distinct quantities of the aldehyde and from this time on there was an increase, the highest concentration being reached about the time breakdown occurred. The accumulation of acetaldehyde was more rapid in late picked pears than in early picked and less mature fruits.

Bartlett pears have been found to contain citric and malic acids. Pears contain about 0.03 mg. of vitamin C in a gram of fresh material. The flesh of the pear contains 4.6 mg. of iron in a kilo of sample, and 1.0 mg. of copper in the same weight.

Pears enjoy an extensive use as a dessert fruit, are canned in large quantities, and are pickled. Many pears are dried.

Total production in 1939: 31,047,000 bu. Average return to the grower: $0.70 a bushel.

(c) *Quince*

The fruit (*Cydonia oblonga*) is apple- or pear-shaped, and hard. With age the skin becomes smooth. The skin is yellow at maturity. The fruit is baked, canned, and used for jellies and preserves.

7. Tropical Fruits (exclusive of Citrus Fruits)

(a) *Avocado*

The avocado (*Persea americana*) is a rather large pear-shaped oily fruit with rough green skin and containing one large seed. It contains 0.28 mg. of vitamin C to the gram of fresh material. It is extensively used as a salad fruit, being known formerly as the alligator pear.

---

[48] C. P. Harley, *J. Agr. Research*, 39, 483 (1929).

Total production in 1939: 10,300 tons. Average return to the grower: $126 a ton.

(*b*) *Banana*

The ripe banana (*Musa sapientum*) is an elongated, fingerlike, angular product, yellow or red in color, with black abortive seeds in the center. It is grown extensively in Central and South America.

TABLE 188

SUMMARY OF CHANGES IN COMPOSITION DURING RIPENING[a]

Stage of ripening	Invert sugar, %	Total sugars, %	Total carbohydrates, %
Green	1.24	7.76	21.51
Green-yellow	1.24	7.76	21.51
Yellow	6 43	17.48	22.12
Yellow-brown	8 55	19.00	20.00
Yellow-brown	11 05	18.26	19.00
Brown	10.90	16 60	16.60

TABLE 189

CARBOHYDRATE CHANGES DURING RIPENING[a]

Stage of ripening	Total carbohydrate, %	Soluble carbohydrate, %	Insoluble carbohydrate, %
Green	26 56	1.30	25.26
Ripe	19 00	17.02	1.98

[a] After E. M. Bailey, *J. Biol. Chem.*, **1**, 355 (1906).

Respiration in the banana was found to be most active when starch hydrolysis was going along at top pace.

Poland and others[49] showed that the principal sugars of the ripe banana are: sucrose, maltose, fructose, and glucose. Maltose is present in very small amounts. The presence of amyl acetate has been demonstrated to be in the volatile oil of the banana. One gram of fresh sample contains 0.01 to 0.14 mg. of vitamin C. The ash contains about 50% of potash. One kilo of the edible part contains 17.6 mg. of iron and 2.1 mg. of copper.

Bananas are extensively used as a dessert fruit, at soda fountains, cooked as a vegetable in fritters, made into flour, flakes, and dried as "banana figs." Banana meal is known also.

U. S. import in 1939: 54,080,000 bunches.

---

[49] G. L. Poland *et al.*, *Food Research*, **2**, 403 (1937).

TABLE 190

PROXIMATE COMPOSITION OF NUTS AND FRUITS[a]

Food	Constituents of the edible portion							
	Water, %	Protein, %	Fat, %	Ash, %	Total carbohydrate, %	Fiber, %	Sugars, %	Acid, %[c]
Almonds	4.7	18.6	54.1	3.0	19.6	2.7	4.4	. .
Apples	84.1	0.3	0.4	0.29	14.9	1.0	11.1	0.47[m]
Summer	86.5	0.3	0.4	0.30	12.5	. . .	9.4	0.70[m]
Winter	83.6	0.3	0.3	0.28	15.5	0.9	11.2	0.46[m]
Applesauce, sweetened	79.8	0.2	0.1	0.2	19.7	0.6	17.6	0.4[m]
Apricots	85.4	1.0	0.1	0.59	12.9	0.6	10.4	1.19[m]
Canned in sirup	77.3	0.6	0.1	0.6	21.4	0.4	. .	. .
Dried	24.0	5.2	0.4	3.5	66.9	3.2	46.0	5.0[m]
Avocados	65.4	1.7	26.4	1.42	5.1	1.8	0.6	. .
Bananas	74.8	1.2	0.2	0.84	23.0	0.6	19.2	0.39[m]
Dried	23.0	3.6	0.3	2.5	70.6	1.7	. .	1.4[m]
Blackberries	85.3	1.2	1.1	0.47	11.9	4.1	6.1	0.91[c]
Canned in sirup	79.2	0.7	0.7	0.3	19.1	2.5	15.6	0.6[c]
Blueberries	83.4	0.6	0.6	0.28	15.1	1.2	9.7	0.67[c]
Canned, juice pack	88.0	0.4	0.4	0.2	11.0	1.0	. .	. .
Brazil nuts	5.3	14.4	65.9	3.4	11.0	2.1	1.5	. .
Butternuts	3.8	23.7	61.2	2.9	8.4	. . .	. .	. .
Cashew nuts,[b] roasted	4.1	19.6	47.2	2.7	26.4	1.0	6.8	. .
Cherries, sour	84.4	1.3	0.5	0.51	13.3	. . .	9.5	1.38[m]
Cherries, sour canned in sirup, red pitted	70.2	0.6	0.1	0.6	28.5	0.2	. .	. .
Cherries, sweet	80.0	1.1	0.5	0.6	17.8	0.4	11.6	0.68[m]
Canned, in sirup, white	78.1	0.6	0.1	0.4	20.8	0.2	. .	. .
Chestnuts[b]	53.2	2.8	1.5	1.0	41.5	1.1	6.4	. .
Citron, candied	18.0	0.2	0.3	1.3	80.2	1.4	. .	. .
Coconut (fresh meat with brown skin)	46.9	3.4	34.7	1.0	14.0	3.2	5.1	. .
Moist, shredded	17.3	3.7	28.6	0.8	49.6	4.2	32.0	. .
Cranberries	87.4	0.4	0.7	0.20	11.3	1.4	4.2	2.36[e]
Cranberry sauce, canned, sweetened	48.1	0.1	0.3	0.1	51.4	0.4	43.0	0.9[c]
Currants, red, white, black	84.7	1.6	0.4	0.61	12.7	3.2	5.7	2.30[c]
Dates	20.0	2.2	0.6	1.8	75.4	2.4	61.2	. .
Figs, fresh	78.0	1.4	0.4	0.64	19.6	1.7	16.2	0.17[c]
Dried	24.0	4.0	1.2	2.4	68.4	5.8	55.0	0.6[m]
Canned in sirup	68.5	0.8	0.3	0.4	30	0.9	28	0.1[c]
Filberts[b]	6.0	12.7	60.9	2.7	17.7	3.4	3.2	. .
Gooseberries	88.3	0.8	0.4	0.39	10.1	2.5	4.2	2.32[c]
Canned in sirup	80.5	0.5	0.2	0.3	18.5	1.5	. .	. .
Grapefruit	88.8	0.5	0.2	0.42	10.1	0.3	6.5	. .
Canned in sirup	85.5	0.5	0.2	0.3	13.5	0.2	. .	. .
Grapes, American	81.9	1.4	1.4	0.45	14.9	0.5	11.5	1.21[m]
Grapes, European	81.6	0.8	0.4	0.46	16.7	0.5	14.9	0.47[m]
Hickory nuts	3.5	13.9	67.4	2.0	13.2	2.2	. .	. .
Huckleberries[d]	. .	. .	. .	. .	. .	. . .	. .	. .
Lemons	89.3	0.9	0.6	0.54	8.7	0.9	2.2	5.07[c]
Limes	86.0	0.8	0.1	0.8	12.3	. . .	0.5	5.9[c]
Loganberries	82.9	1.0	0.6	0.52	15.0	1.4	6.0	2.18[c]
Canned, juice pack	86.0	1	0.1	0.4	12.5	2	. .	. .
Macadamia nuts	3.1	8.7	71.4	1.7	15.1	2.5	2.7	. .

TABLE 190 (*Continued*)

PROXIMATE COMPOSITION OF NUTS AND FRUITS[a]

Food	Constituents of the edible portion							
	Water, %	Protein, %	Fat, %	Ash, %	Total carbohydrate, %	Fiber, %	Sugars, %	Acid, %[c]
Muskmelons, all	92.7	0.6	0.2	0.6	5.9	0.5	5.4	..
Honeydew, casaba Spanish	90.6	0.6	0.2	0.6	8.0	0.5	7.0	..
All others plus cantaloupe	94.0	0.6	0.2	0.6	4.6	0.6	4.2	..
Nectarines	82.9	0.5	0.1	0.5	16.0	0.4	11.8	1.15[m]
Oranges, fresh	87.2	0.9	0.2	0.47	11.2	0.6	8.8	0.68[c]
Canned	83	0.8	0.2	0.5	15.5	0.5	..	..
Oranges, Tangerines	87.3	0.8	0.3	0.66	10.9	1.0	8.7	0.93[c]
Peaches	86.9	0.5	0.1	0.47	12.0	0.6	8.8	0.64[m]
Canned in sirup	80.9	0.4	0.1	0.4	18.2	0.4	..	..
Dried	24	3.0	0.6	3.0	69.4	3.5	51.0	3.0[m]
Peanuts, roasted in shell, Virginia	2.6	26.9	44.2	2.7	23.6	2.4	..	..
Pears	82.7	0.7	0.4	0.39	15.8	1.4	8.9	0.29[c]
Bartlett	83.5	0.4	0.4	0.3	15.4	...	8.3	0.32[c]
Canned in sirup	81.1	0.2	0.1	0.2	18.4	0.8	..	..
Dried	24	2.3	0.4	1.7	71.6	6.1	36.0	1.5[m]
Pecans	3.0	9.4	73.0	1.6	13.0	2.2	3.9	..
Pineapple	85.3	0.4	0.2	0.42	13.7	0.4	11.9	0.72[c]
Canned in sirup	78.0	0.4	0.1	0.4	21.1	0.3	18.6	0.8[c]
Pinenuts, Pignolias	4.9	31.2	48.4	4.3	11.2	1.0	4.3	..
Pistachio nut	5.6	19.6	53.2	3.0	18.6	2.2	6.1	..
Plums	85.7	0.7	0.2	0.51	12.9	0.5	8.3	1.6[m]
Damsons	78.8	..	..	0.67	13.5	0.4	8.7	2.02[m]
European type excluding damsons	86.1	0.7	0.1	0.47	12.6	...	8.5	1.46[m]
Canned in sirup	78.6	0.4	0.1	0.5	20.4	0.3	..	..
Pomegranate	81.0	0.6	0.2	0.5	17.7	0.3	13.3	1.05[c]
Quince	85.3	0.3	0.1	0.38	13.9	1.8	6.3	0.87[m]
Raspberries, red	83.4	1.1	0.6	0.47	14.4	2.8	7.2	1.34[c]
Black	80.7	1.5	1.6	0.65	15.6	3.5	7.9	1.16[c]
Strawberries	90.0	0.8	0.6	0.50	8.1	1.2	5.3	1.09[c]
Canned in sirup	70.8	0.5	0.2	0.5	28.0	0.7	..	..
Walnuts, black	2.7	18.3	58.2	2.1	18.7	1.9	..	..
Walnuts, English	3.3	15.0	64.4	1.7	15.6	2.1	..	..
Watermelon	92.1	0.5	0.2	0.27	6.9	0.6	6.0	0.03[m]

---

[a] Charlotte Chatfield and Georgian Adams, U. S. Dept. Agr., *Circ.* **549** (1940).

[b] Starch in cashew nuts = 10.7%. Starch in chestnuts = 14.8%. Starch in filberts = 1.6%.

[c] When [c] appears after the figure for acid, citric acid is indicated. Malic acid is indicated by [m].

[d] See blueberries.

(*c*) *Date*

The date (*Phoenix dactylifera*) is, botanically, an elongated berry, wrinkled on the surface in the dried condition as it is customarily found on the market, and either light brown or dark brown in color. The Deglet Noor dates as grown in California are nonsticky unless they have been hydrated. Large amounts are grown in Asia Minor and North Africa.

It has been stated that sucrose is the principal sugar of the Deglet Noor date but that inversion in the fruit before and after harvesting causes one-fifth to one-fourth of the sugar to be converted to invert sugar. Dried dates as sold contain 50.7 mg. of iron and 3.8 mg. of copper to the kilo.

Total production in 1939: 2596 tons. Average return to the grower: $126 a ton. Total production in 1940: 6031 tons. Average price: $116 a ton. U. S. import in 1939: 22,546 tons.

(*d*) *Fig*

The fig (*Ficus carica*) is a pear-shaped receptacle on a short stalk. The seeds are on the inside of the walls of the receptacle. Considerable variation is encountered with regard to such factors as size, color, form, and seeds. The seeds are the true fruits of the fig tree. It is grown in large quantities in California, where it was first introduced, and in the southern States. The Mediterranean region is especially well suited to fig culture.

Citric and acetic acids have been found in figs. The ash of the fig is high in potash. Dried figs contain 39.6 mg. of iron and 3.5 mg. of copper to the kilo.

Figs are used as a dessert fruit, are canned, dried, and preserved. A sirup of figs is used medicinally.

Total production in 1939: 88,490 tons, on the fresh basis. Average return to the grower: $28.82 a ton. Production for California in 1939: 26,000 tons (dry basis) of dried figs. U. S. import during this period: 2323 tons of dried figs.

(*e*) *Pineapple*

The fruit (*Ananas sativus*) is somewhat cylindrical, tapering at the top and crowned with a tuft of stiff leaves. It is multiple, and each individual fruit is roughly hexagonal in outline. It grows in Hawaii, Florida, Puerto Rico, Cuba, and other parts of the world.

Respiration studies conducted on the Red Spanish variety, using a kilo of sample, gave an evolution of 104 mg. of carbon dioxide in 1 hr. at 31.2° C. At 1.2° C. the value for carbon dioxide was 7 mg. in an hour, for 1 kilo of sample.

The colors in the pineapple flesh were found to be carotene and smaller amounts of xanthophyll. The ester distillation method showed that the total acid was made up of 87% citric and 13% *l*-malic acids. It contains 0.25 to 0.60 mg. of vitamin C to the gram of sample. The ash of the pineapple is comparatively rich in potash. One kilo of the fresh edible material is known to contain 3.2 mg. of iron and 0.7 mg. of copper.

Pineapple is used as a dessert fruit. Large quantities are canned in slices, fingers, chunks, and grated. A considerable quantity of pineapple juice is also packed. Pineapple tidbits are canned with fruit cocktail.

In 1939, 536,327,000 lbs. of pineapple were canned, 311,677,000 lbs. of juice were packed, and 123,000 boxes of 20 pineapples to the box were produced in Hawaii.

(*f*) *Pomegranate*

The pomegranate (*Punica granatum*) is very roughly spherical. Many angular seeds are crowded into the interior with the bulky placental tissue. The rind is thin and tough. The exterior is usually colored red, and the juice is of this same color. Citric acid has been shown to be the only acid of the pomegranate. The edible part contains 11.7 mg. of iron to the kilo, fresh basis. It is used as the fresh fruit, for the preparation of juice, and in the manufacture of true grenadine.

Chapter XX

CARBOHYDRATE AND SUGAR FOODS

ED. F. DEGERING

Lafayette, Indiana

Chemical elements in a variety of combinations are the ultimate building units of the human body. Since the human body is a dynamic system, these elements are being used up continuously and replacement is essential if health is to be maintained. A diet which supplies all of the essential elements in requisite amounts, for building and replacement needs, is commonly known as an adequate or normal diet.

With a very limited supply of food at their disposal, our ancestors who roamed the ranges and the forests could depend rather safely on their appetites as a guide in the selection of their diets. They were not concerned with surpluses. Modern civilization, however, is suffering from both too much food and too many varieties of food. Something over 40,000 manufacturers of foods in the United States are introducing new products at the rate of about two a day. Man, consequently, has become finicky with respect to his diet, and the appetite can no longer serve as a proper criterion in the selection of food.

Food, as commonly defined, consists of carbohydrates, fats, proteins, vitamins, mineral salts, water, and oxygen. The first of these will be considered in detail in this chapter, whereas the other factors are considered elsewhere in this treatise (see pp. 88, 110, 229, and 270).

The carbohydrate content of the edible portion of some of the common foods is given in Table 191. A glance at this table together with the fact that the average American consumed, in some way or another, about 100 lbs. of sucrose in 1941, leads one to the conviction that the role of the carbohydrates and sugars in the dietary of the normal individual is indeed an important one.

I. HISTORICAL DEVELOPMENT

Sugar was not always as plentiful as it is today. It was once a luxury for kings. The growth and development of the inadequate and tiny sugar production of the past into the overproduction of today is another great chemical and technological triumph. When the housewife in her round of shopping buys refined sugar at its current low price, she is unwittingly benefiting from the master thought and arduous labors that have gone into a modern industry whose humble beginnings date back into the dim and distant past.

About 1000 B.C., in writing of the creation of the world, Moses penned the lines "And God said, Behold, I have given you every herb bearing seed, which is upon the face of all of the earth, and every tree, in the which is the fruit of a tree yielding seed to you it shall be for meat" (*Gen. 1: 29*). Instinct led man to cherish the sweet fruits which he gathered. Gradually he learned to press them to obtain sweet drinks.

Table 191

Per Cent Carbohydrate of Edible Portion of Foods

Almonds	17.3
Apples	14.2
Apricots	13.4
Artichoke, French	12.0
Asparagus	3.9
Avocado	7.4
Bananas	23.0
Barley, entire	72.8
pearled	77.8
Beans, dried	59.6
Lima, dried	65.9
Lima, fresh	23.5
snap or string	7.7
Beef, dried, salted, and smoked	0.4
heart	1.0
liver	1.7
Beets, cooked	7.4
fresh	9.6
Boston crackers	71.1
Brazil nuts	7.0
Bread, Boston brown	54.0
graham	52.1
rolls, water	54.2
toasted	61.2
white, homemade	53.3
white, average	53.1
whole wheat	49.7
Broccoli	5.5
Buckwheat flour	77.9
Buttermilk	3.9
Butternuts	3.5
Cabbage	5.3
Carrots	9.3
Cauliflower	4.9
Celery	3.7
Chard	4.4
Cheese, Cheddar type	0.6
Cherries	14.8
Chestnuts, fresh	42.1
Chocolate	30.3
Cocoa	37.7
Consomme, canned	0.4
Corn, sweet, fresh	20.5
Corn meal	75.4
Cowpeas, dried	60.8
green	22.7
Crackers, butter	71.6
cream	69.7
graham	73.8
soda	73.1
Cranberries	11.3
Cream, light	4.5
medium	4.0
"whipping"	3.5
Cucumbers	2.7
Currants	12.7
dried, Zante	74.2
Dandelion greens	8.8
Dates, dried	78.4
Eggplant	5.5
Farina	76.3
Figs, dried	74.2
Flour, rye	78.7
wheat, Calif., fine	76.4
wheat, entire	71.9
wheat, graham	71.4
wheat, patent baker's grade	72.7
wheat, av. high and medium	75.1
wheat, av. low grade	71.2
Grapes	19.2
Grapefruit	10.1
Hominy	79.0
Honey	81.2
Huckleberries	15.1
Kale	7.2
Kohlrabi	6.7
Koumiss	5.4
Lemon	8.7
Lettuce	2.9
Liver, beef	1.7
Lobster, whole	0.4
Macaroni	74.1
Macaroons	65.2
Marmalade, orange	84.5
Milk, cond., sweet	54.1
skimmed	5.1
whole	5.0
Molasses, cane	69.3
Muskmelons	9.3
Oatmeal	67.5
Okra	7.4
Olives, green	11.6
ripe	4.3
Onions, fresh	9.9
Oranges	11.6
Oysters	3.7
Parsnips	18.2
Peaches, fresh	12.0
Peanuts	24.4
Pears, fresh	15.8
Peas, dried	62.0
green	17.7
Peppers, green	5.7
Persimmons, native	33.5
Pies, apple	42.8
custard	26.1
lemon	37.4
mince	38.1
squash	21.7
Pineapples, fresh	13.7
Pistachios, shelled	16.3
Plums	12.9
Pomegranates	20.9
Pork sausage	1.1
Potato chips	46.7
Potatoes, white, raw	19.1
Prunes, dried	73.3
Pumpkins	7.3
Radishes	4.2
Raisins	76.1
Raspberries, red	14.4
black	15.6
Rhubarb	3.8
Rice	79.0
Sausage, Bologna	0.3
Shad roe	2.6
Shredded wheat	77.9
Squash	9.0
Strawberries	8.1
Succotash, canned	18.6
Sugar	100.0
Sweet potato	27.9
Tomatoes, fresh	3.9
canned	4.0
Turnips	7.1
Vegetable soup, canned	.5
Walnuts, Calif. or Eng.	13.0
black	11.7
Watercress	3.3
Watermelons	6.7
Wheat	75.5
Zwieback	73.5

Still later he learned to concentrate some of these solutions, especially that obtained from the sugar cane, to give a sweet sirup. Finally, about 300 to 600 A.D., crystalline sugar was isolated.

When Alexander the Great invaded India in 327, his scribes reported finding the people chewing "a marvelous reed which produced a kind of honey without any aid from bees." The culture of this product spread into Persia, where developments with respect to both growth and refining were recorded. When the Arabs overran Persia in 640, they recognized the importance of this new industry. They, accordingly, encouraged the production of sugar cane, built large hydraulic mills for processing the cane, and imposed heavy taxes on both the equipment and the product. Many of the provinces paid their annual tribute in crystalline sugar. As a relic of the contribution made by the Arabs to the culture of sugar cane and the refining of the juice, is the name of the product, sugar, which comes from the Arabic term, *shukkar*.

The cane-sugar industry was introduced by the Arabs into Egypt, where further advances were made. Here it was found that better clarification of the juice from the cane was obtained by the addition of lime and plant ashes. This resulted in better crystallization. The Egyptians learned, furthermore, to produce large crystals which are now known as rock candy. During this period of development of the industry it was found that the juice can be clarified by the addition of lead acetate, but the practice led to a number of fatalities and was soon prohibited.

The industry spread from Egypt throughout North Africa, the islands of the Mediterranean, and into Spain. At about the same time the crusaders acquired the taste for sugar in their journeys throughout the Near East, and those who returned to northern Europe brought back, among other things, a sample of sugar. This stimulated the demand, and by the year 1200 sugar was a standard commodity in southern France and well known in Germany and the other accessible parts of Europe.

On the second voyage of Columbus in 1493, he brought sugar cane to San Domingo and thus introduced to the Western World what was to become a first-rate industry. Systematic culture of sugar cane began in the New World in about 1515 and flourished beyond the fondest expectations. In less than 100 years after the discovery of America, sugar was one of the most valuable items of export by such countries as Brazil, Cuba, and Mexico. Fleets carried the crude sugar to such ports as Antwerp and Lisbon, where it was refined and distributed throughout Europe. The introduction of chocolate, coffee, and tea as standard commodities increased the demand for sugar. With a marked increase in consumption, there was a corresponding increase in production and a consequent decrease in price until sugar is now one of the cheapest sources of biological energy.

Real improvements in the technique of sugar refining were introduced with the development of modern chemistry during the middle of the 18th century. About this time, moreover, a professor of chemistry by the name of A. S. Marggraf (1709–1782) made a major contribution to the chemistry and technology of sugar in that he discovered a new source for this important commodity. Marggraf, in 1747, as Director of the Berlin Academy of Science, reported that he had examined a number of sweet-tasting plants and found that several of them contained a sugar identical with that extracted from sugar cane. Of the various plants examined, the beet (now known as the sugar beet) was particularly promising. "It is evident," said Marggraf, "that this sweet 'salt' can be prepared in our region just as well as where the sugar cane grows." Europe had gone Chemurgic. Every peasant was to produce his own sugar.

Although Marggraf outlined in some detail the isolation and purification of sugar from beets, the commercial development fell to the lot of his student and successor, Franz Achard (1753–1821). In 1799 Achard addressed a petition to Frederick William III, the Prussian ruler, asking that he might "be enabled to enjoy the fruits of his labor, prosecuted for fifteen years with great diligence and cost." He requested that he be given "the exclusive privilege for ten years for the manufacture of native sugar and the grant of sufficient land, whose soil would be adpatable to beetroot culture." In due time the grant was approved and $37,500 was appropriated for a factory in Silesia, which started production in 1802. This plant was soon producing 6% of raw sugar and 3% of molasses from the freshly harvested beets.

But this humble beginning of a new industry soon felt the effect of the Napoleonic wars which swept over Prussia, and progress in the culture of the beets and the technique of processing them was very slow. In 1809 the factory of Achard was burned to the ground, leaving the owner heavily in debt and with a family in distress.

The subsequent continental blockade raised the price of sugar to seven francs per pound, which forced Napoleon to subsidize the French beet-sugar industry to the extent of 1,000,000 francs. With the fall of Napoleon and the subsequent withdrawal of the subsidy, the beet-sugar industry of France was unable to compete with the sugar-cane industry of the New World. As early as 1828 in France, however, and by 1836 in Germany, the sugar-beet industry was firmly established and was successfully competing with the product of the sugar-cane industry.

During this period of development of the sugar-beet industry in Europe, marked progress had been made in the sugar-cane industry. Among these were such items as concentration in thin layers, the use of clarification pans fitted with steam coils, bleaching with indigo or other chemical, and the introduction of bronze and copper pumps to displace the use of ladles. The concentration with steam under reduced pressure was introduced by Howard in 1813, the use of animal charcoal as a decolorant and purifier was first used in New Orleans in the same year, and the juice lifts were developed by Dubrunfaut in 1819. It is of interest to note that the use of animal charcoal as a purification absorbent was discovered accidently by two French apothecaries who were compounding a shoe polish.

The price curve for sugar is characteristic of a major chemical industry. Listed at $3.30 per pound in 1260, the price of sugar declined to $0.55 per pound in the latter part of the 18th century and has since shown a decline trend to its current low level.

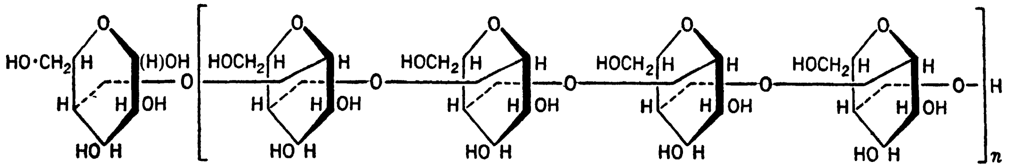
Sugar cane is currently produced in Australia, Brazil, China, Cuba, Egypt, India, Jamaica, Japan, Peru, Philippine Islands, other tropical countries, and some of the southern States. In the United States the sugar-beet industry has assumed commercial production in California, Colorado, Indiana, Kansas, Michigan, Montana, Nebraska, South Dakota, Utah, and Wisconsin, and, on a small scale, in some of the other states.

II. CARBOHYDRATES USED AS FOODS

The carbohydrate materials which are utilized by the human organism, listed in the approximate order of decreasing complexity, are: cellulose, hemicellulose, inulin, starch, glycogen, dextrins, stachyose, raffinose, lactose, maltose, sucrose, mannose, fructose, glucose, and galactose. Each of these will be considered in some detail with respect to structure and commercial production.

1. Cellulose

Cellulose is the fibrous or woody material which forms the cell walls of all vegetable material. Its building blocks are *β*-*D*⁺-glucose units, linked together in the 1,4-positions to give a continuous chain. (Compare formula I.) Inasmuch as the digestive enzymes and ferments are not capable of attacking this *β*-glucosyl linkage, as it occurs in cellulose, this carbohydrate is not utilized as such by the human organism. Since, however, its building units are glucose, which is one of the most important of the sugar foods, the molecule may be degraded *in vitro* and then utilized *in vivo*. This is exactly what happpened on the Continent during the first World War and it is altogether likely that waste cellulose material may utimately be utilized effectively by degradation to *D*⁺-glucose.



Formula I.—Diagrammatic representation of the cellulose molecule.

2. Hemicellulose

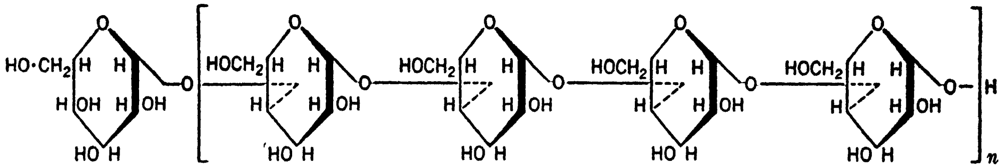
Hemicellulose is a term used to describe certain substances which resemble cellulose in that they belong to the walls rather than the contents of plant cells, but resemble starch in that they are readily hydrolyzed by acids. Unlike starch, however, they yield sugars other than *D*⁺-glucose such as arabinose, xylose, galactose, or mannose or combinations of these. The hemicelluloses may be utilized to some extent by the human organism.

3. Inulin

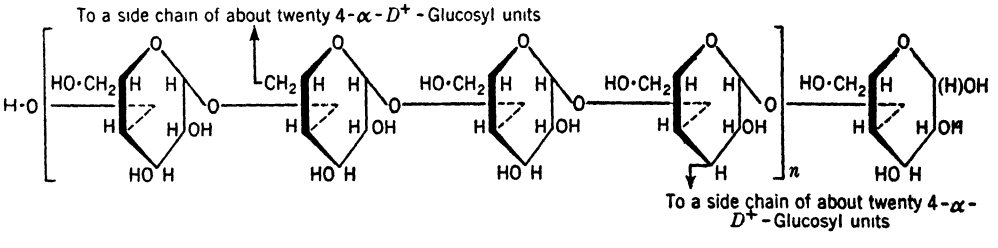
Inulin occurs in certain lichens, chicory root, dahlia bulbs, dandelion roots, and Jerusalem artichokes. Upon hydrolysis, it yields D^--fructose. Although not produced extensively, inulin does offer a source for the commercial production of D^--fructose, which is the sweetest of the simple sugars.

4. Starch

Starch is the form in which most plants store their reserve carbohydrate, and the form in which carbohydrate material is most commonly utilized by the higher forms of life. Like cellulose, it is built up of D^+-glucose units, but unlike cellulose the linkage between the units is of the α- rather than the β-type. Starch, moreover, is composed of two relatively different types of molecules. The one is of a continuous chain variety (formula II), thus resembling cellulose, whereas the other is of the branched chain type (formula III). Ordinary corn starch contains about 30% of the variety indicated by formula II and 70% of that indicated by formula III, whereas waxy-maize corn starch is essentially of the type indicated by formula III.



Formula II.—1,4-Linkage of α-D^+-glucosyl units in the formation of a continuous-chain starch molecule.



To a side chain of about twenty 4-α-D^+-Glucosyl units

To a side chain of about twenty 4-α-D^+-Glucosyl units

Formula III.—1,4-Linkages of α-D^+-glucosyl units, with periodic branching, to give a branched chain starch molecule.

Starch serves as a very important source of food for both human beings and the lower forms of life. It is used extensively in its naturally occurring forms such as beets, carrots, corn, oats, parsnips, potatoes, rice, wheat, and similar products as well as in various stages of hydrolysis and refinement such as dextrins, corn sirups, and crystalline glucose. The α-type linkage in the starch molecule is readily attacked by the digestive enzymes, and the molecule is degraded through hydrolysis to its simple unit, D^+-glucose, before being absorbed by the blood stream.

5. Glycogen

Glycogen, since it plays much the same role in animals that starch does in plants, is frequently referred to as animal starch. It is an amorphous, odorless, tasteless, white powder, which apparently dissolves in cold water to give an opalescent colloidal solution. Such solutions, although they are not cleared by repeated filtrations, readily lose their opalescence by a change in the hydrogen-ion concentration. Glycogen may be recovered from such solutions by precipitation with alcohol. The Wagner reagent (iodine in concentrated potassium iodide solution) reacts with glycogen to give a yellowish-brown, reddish-brown, or deep red coloration.

Glycogen is found in liver tissue (about 10%), in muscle tissue, and in small amounts in the blood, brain, kidneys, pancreas, spleen, and in a large number of fungi. The total amount and distribution of the glycogen in the body will vary somewhat depending on the comparative state of activity of the individual. Glycogen is built up of D^+-glucose units, but its rate of hydrolysis in the animal organism is much higher than is that of starch.

6. Dextrins

Dextrins are the partial degradation products of starch. They may be produced by the action of acids, enzymes, or heat upon starch. Complete degradation of the dextrins in turn yield D^+-glucose. Dextrins occur in honey, in fruit and vegetable juices, in many plants, and as probable intermediates in the metabolism of starch. Malt diastase, for example, reacting for some time in fairly concentrated solution upon starch, yields about four parts of maltose to one part of dextrin. The dextrins, according to the work of Rettger,[1] afford a favorable medium for the growth of *Lactobacillus acidophilus* in the intestinal tract. The dextrins, since they are hydrolytic products of starch, should be and are much more soluble in water than are the starches.

7. Stachyose

Stachyose is a nonreducing tetrasaccharide, which is composed of one unit of fructose, two units of galactose, and one unit of glucose, that is, it is galactosyl-galactosyl-glucosyl-fructoside. On hydrolyis by enzymes or acetic acid D^--fructose and manninotriose are formed. It is believed that it is utilized to a limited extent at least by the animal organism.

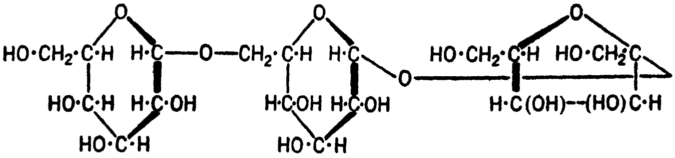
8. Raffinose

Raffinose is a nonreducing trisaccharide, which is composed of one unit of fructose, one unit of galactose, and one unit of glucose. Specifically it is designated as 6-α-

---

[1] L. F. Rettger *et al.*, *Lactobacillus Acidophilus and Its Therapeutic Applications.* Yale Univ. Press, New Haven, 1935.

D^+-galactosyl-α-D^+-glucosyl-β,γ-D^--fructoside. It may be hydrolyzed to give one unit of galactose and one unit of sucrose, or one unit of fructose and one unit of melibiose. If the intestinal hydrolysis is such as to yield the former set of derivatives, it is completely utilized in the digestive tract. If, however, hydrolysis yields fructose and melibiose, the fructose alone is completely utilized. The accepted configuration of raffinose is shown in formula IV.

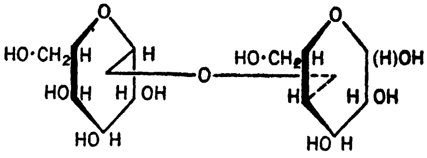


Formula IV.—Configuration of raffinose (6-α-D^+-galactosyl-α-D^+-glucosyl-β-γ-D^--fructoside).

9. Lactose

Lactose, as shown in formula V is built up of a galactose and a glucose residue. Upon hydrolysis, either acidic or enzymatic, the end products are D^+-galactose and D^+-glucose, both of which are readily assimilated by the animal organism.

Lactose, because it occurs in milk to the extent of 4 to 7%, is sometimes referred to as milk sugar. Experimental evidence indicates that this sugar is formed in the mammary gland from the D^+-glucose supplied by the blood. On a comparative basis with sucrose at 100, the sweetness of lactose is only 16. The solubility of lactose in cold water is 17 parts per hundred at 20° C., whereas that of sucrose is 179 parts per hundred at 0° C. This may account in part at least for the fact that lactose is less readily fermented than is sucrose, and is also less irritating to the membrane of the stomach.



Lactose, 4-β-D^+-Galactosyl-D^+-Glucose

Formula V.

Matthews[2] has suggested that the lactose of milk may serve as the source for the galactose residue for the synthesis of the galactosides of the brain and the nerve tissues of the rapidly growing young mammal. Lactose, according to Rogers and coworkers,[3] is particularly desirable for the maintenance of good intestinal conditions because it is conducive to the proper growth and development of *L. acidophilus*. When solutions of lactose are allowed to crystallize above 90° C., the β-lactose or 4-β-D^+-galactosyl-β-D^+-glucose is formed. This product is more soluble in water and sweeter than is the α-variety and has been marketed to a limited extent.

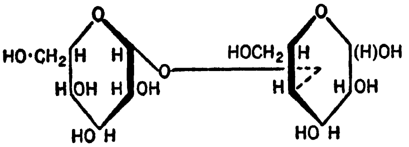
---

[2] A. P. Matthews, *Physiological Chemistry*. 5th ed., Wm. Wood, Baltimore, 1930.

[3] R. Rogers *et al.*, in 1935 and subsequently. See H. C. Sherman, *Chemistry of Food and Nutrition*. Macmillan, New York, 1937, pp. 20, 111.

10. Maltose

Maltose is an important component of germinating cereals, malt, and malt products inasmuch as it is formed by the action of the amylases on starch. It is, morever, an intermediate in the acidic or enzymatic hydrolysis of starch. During digestion, maltose is formed by the action of the ptyalin of the saliva or the amylopsin of the pancreatic juice upon either dextrin or starch. As indicated by the formulation in formula VI, maltose is built up from D^+-glucose units and is correctly described as 4-α-D^+-glucosyl-D^+-glucose. On a weight basis it is about one-third as sweet as cane sugar. Its solubility in water is 108 parts per hundred. When injected directly into the blood stream, maltose appears to be utilized better than is either lactose or sucrose. Maltose is hydrolyzed by either acid or enzymes to yield two units of D^+-glucose.

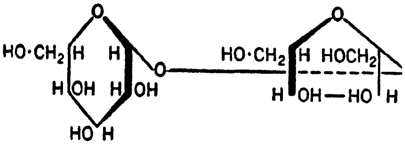


Maltose, 4-α-D^+-Glucosyl-D^+-Glucose

Formula VI.

11. Sucrose

Sucrose occurs in almonds, coffee, flowers, honey, maple sap, sugar beet, sorghum cane, sugar cane, walnuts, and in small amounts, associated with D^+-glucose or D^--fructose or both, in most plants. The important commercial sources are the sugar beet, the sorghum and sugar canes, the sugar maple, and the sugar palm. The solid matter of certain fruits, such as the pineapple, and of certain vegetables, such as the carrot, is as much as 50% or more sucrose. The consumption of sucrose in the United States has increased from about 68 lbs. per capita in 1900 to something over 100 lbs. in 1941. This means that the average American is taking approximately one-seventh of his total calories in the form of sucrose.[4] This is particularly significant when one recalls that in the pre-sugar era the normal individual was consuming about one-seventh more mineral elements, proteins, and vitamins in order to supply his requirement for energy. Sherman even goes so far as to question whether or not we would be better off "if a part of the producer's land and labor, and of the consumer's money, now devoted to sugar, were devoted instead to increasing the production and consumption of foods which together with their calories furnish us also other nutrients needed to balance our dietary."



Sucrose, α-D^+-Glucosyl-β-γ-D^- Fructoside

Formula VII.

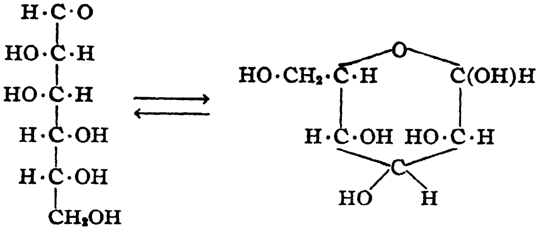
Sucrose is very readily hydrolyzed by either acid or the enzyme, sucrase, which may be supplied by yeast or the intestinal juice. The hydrolytic products, as indicated by the formulation in formula VII, are D^+-glucose and D^--fructose, both of which are readily metabolized by the animal organism. D^--Fructose has the distinction of being the sweetest known sugar, its sweetness coefficient being 173.3 on the basis of 100 for sucrose. Since the sweetness coefficient for D^+-glucose is 74.3, it is apparent that the sweetness of sucrose is increased from 100 to about 123 on a weight basis when it is completely hydrolyzed to its components.

---

[4] H. C. Sherman, *Chemistry of Food and Nutrition*. Macmillan, New York, 1941, p. 15.

12. Mannose

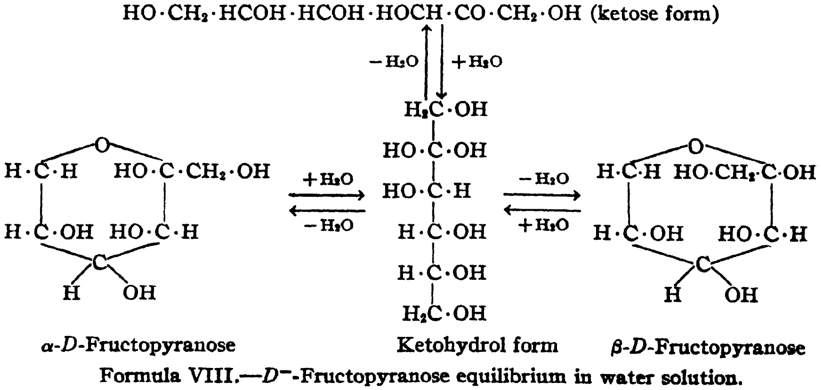
Mannose, formulated below, is a constituent of a number of proteins and glycosides. Like D^--fructose and D^+-galactose, it is likely that D^+-mannose is converted to D^+-glucose and then synthesized into glycogen for storage or else metabolized directly. Because of its limited availability and because of limited information to date relative to its metabolism, further consideration will not be given to mannose as a food.



D^+-Mannose equilibrium in aqueous solution.

13. D^--Fructose

D^--Fructose is an ingredient of honey, is the essential constituent of inulin, and is one of the building units of the disaccharide sucrose and the trisaccharide raffinose. It is the most important of the naturally occurring ketose sugars. Although D^--fructose occurs in the furanoid ring as a building unit in sucrose, the equilibrium shown in formula VIII is doubtless the best representation of a water solution of this sugar. Experimental data indicate that D^--fructose is metabolized somewhat more readily



α-D-Fructopyranose   Ketohydrol form   β-D-Fructopyranose

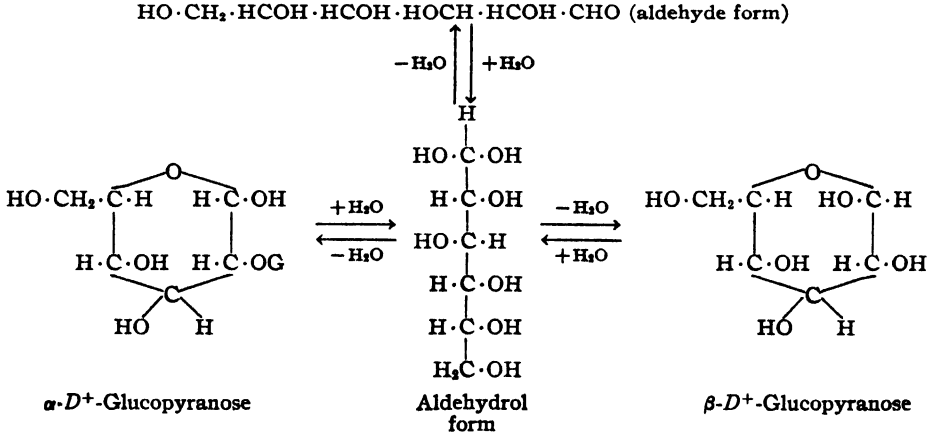
Formula VIII.—D^--Fructopyranose equilibrium in water solution.

than is D^+-glucose. Inasmuch as D^--fructose does not occur in the blood stream, it is believed that it is converted directly into glycogen and then hydrolyzed to yield D^+-glucose as the energy requirements of the body may demand.

14. D^+-Glucose

D^+-Glucose (dextrose, corn sugar, grape sugar, diabetic sugar, starch sugar) is the ultimate hydrolytic product of both cellulose and starch, and is an integral building unit in practically all of the oligosaccharides. It commands, accordingly, a very important place in any consideration of the metabolic utilization of the carbohydrates and sugars.

D^+-Glucose is widely distributed in nature, particularly in ripe fruits, flowers, honey, leaves, roots, sap, urine of diabetics, and in small amounts in normal urine and in the blood (about 0.1%). As much as 20% of the total weight of ripe grapes is glucose. As previously noted, D^+-glucose is the final hydrolytic product of cellulose, hemicellulose, starch, glycogen, dextrins, and maltose, and is among the hydrolytic products of lactose and sucrose. "It is estimated that (under ordinary conditions) over half of the energy manifested in the human body is in this sense derived from the oxidation of glucose."[5] The postulated equilibrium relations for a water solution of D^+-glucose is shown in formula IX. Whereas D^+-glucose exists primarily in the form of the pyranose ring structure, Irvine and Robertson suggest that we must always consider the possibility of the 1-2, 1-3, 1-4, 1-5, and 1-6 oxide rings.[6] To date there is no evidence for anything but the 1,5-glucopyranose ring in the carbohydrates that have been studied critically, but this does not mean that an equilibrium of all of these possible systems might not be the best representation for a water solution of D^+-glucose (see formula IX).



Formula IX.—D^+-Glucopyranose equilibrium in water solution.

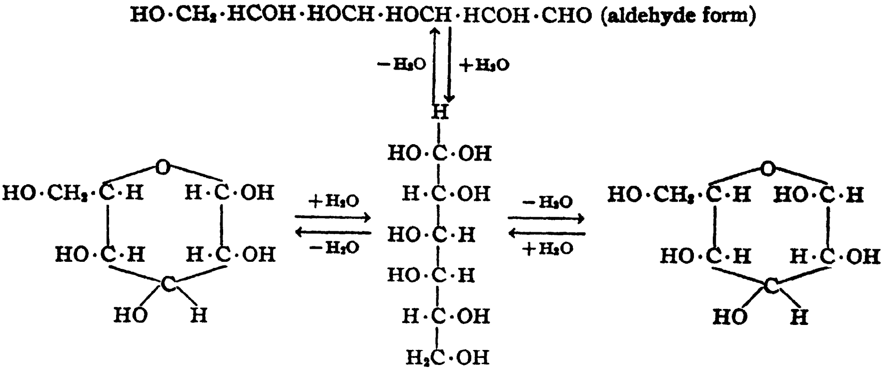
---

[5] H. C. Sherman, *Chemistry of Food and Nutrition*. Macmillan, New York, 1941, p. 11.

[6] J. C. Irvine and G. J. Robertson, *Ann. Rev. Biochem.*, 4, 59 (1935).

15. D^+-Galactose

D^+-Galactose is the essential building unit of agar-agar, and is one of the building units of both lactose and raffinose. Galactose is, moreover, a minor constituent of a number of proteins. It is believed that this sugar plays an important role in the physiology of the cerebrosides of the brain. A potential commercial source of D^+-galactose is the hydrolysis of the galactans of the mountain larch. The equilibrium system for a water solution of D^+-galactose is given in formula X.

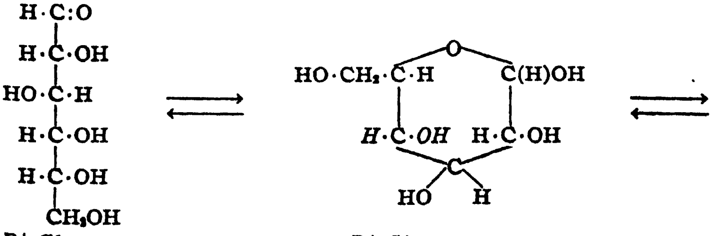


Formula X.—D^+-Galactopyranose equilibrium in water solution.

What appears to be a physiological anomaly is the conversion of D^+-galactose into D^+-glucose for synthesis to glycogen in the liver, and the conversion of D^+-glucose to D^+-galactose for synthesis of lactose in the mammary glands.[7] These transformations involve a type of Walden inversion, which is readily effected *in vitro* in certain cases and may well be very readily effected *in vivo* in this particular instance. The transformation may occur as the result of a special type of enzymatic action in the particular cells concerned (see formula XI).

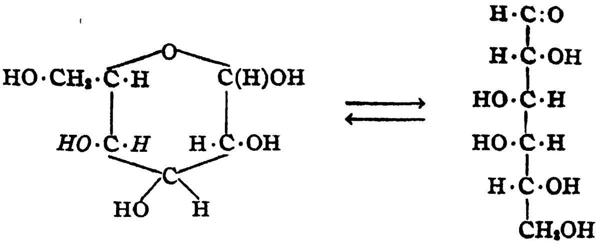
The conversion of one sugar into another before assimilation by the animal organism may not be too far removed from the *in vitro* effect of mild alkali on the simple sugars. D^+-Glucose, D^+-mannose, or D^--fructose, for example, gives rise to the system.

D^+-glucose $\rightleftarrows$ D^+-mannose $\rightleftarrows$ D^--fructose $\rightleftarrows$ other sugars and intermediates



---

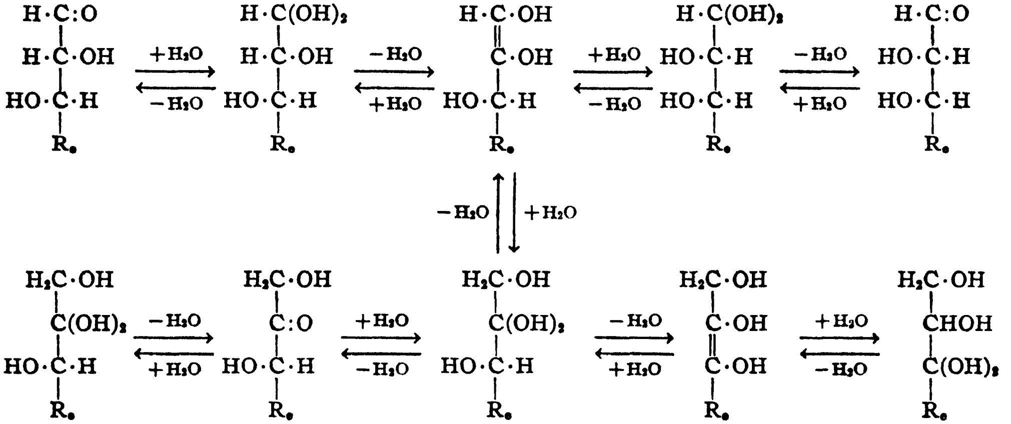
[7] H. J. Deuel, Jr., *Physiol. Revs.*, 16, 173 (1936).



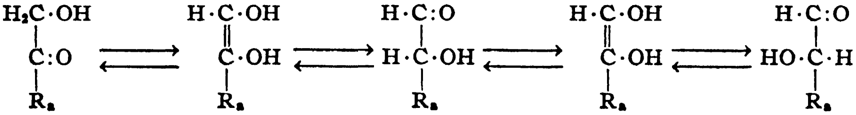
D^+-Galactopyranose D^+-Galactose

Formula XI.—D^+-Glucose-D^+-galactose interrelationship.

Nef[8–10] postulated that this equilibrium results from the formation of an enediol as an intermediate by the addition and subsequent loss of water, as:



Whereas this does explain the interconversion of one sugar into another, the change can be explained equally well by merely assuming a migration of a proton as indicated by the system



Either of these mechanisms, or some other one, under the influence of a biochemical catalyst may account for the ready interconversion of D^--fructose and D^+-galactose (and probably D^+-mannose) into D^+-glucose before final utilization by the animal organism.

---

[8] Ed. F. Degering *et al., An Outline of Organic Chemistry.* Barnes and Noble, New York, 1942, p. 159.

[9] Ed. F. Degering *et al., Fundamental Organic Chemistry.* Swift & Co., Cincinnati, 1942, p. 344.

[10] Ed. F. Degering *et al., An Outline of Organic Nitrogen Compounds.* Purdue Univ., Lafayette, 1942, pp. 95, 108, 112. *An Outline of the Chemistry of the Carbohydrates*, Purdue Univ., Lafayette, 1941, p. 138.

III. THE DIGESTION OF THE CARBOHYDRATES AND SUGARS

In the digestion of carbohydrate material the first attack, particularly in the case of the dextrins and starches, is by the saliva which is secreted by the submaxillary, sublingual, and parotid glands. This digestive secretion is a frothy, slightly opalescent, viscous fluid, which contains many substances. Its normal flow may be stimulated by a variety of factors. Dry foods, as a rule, call for a greater secretion of saliva than do moist foods. Chemical agents as acids, flavored substances, and salts both initiate and stimulate salivary secretion. Even mechanical disturbances such as food, paraffin, sand, or other solid particles, induce the flow of this secretion. It is estimated that the normal individual secretes about 1500 ml. of saliva per day. Of 610 specimens studied, the *p*H of the saliva ranged from 5.75 to 7.05. It is about 99.42% water. Of the 0.58% of solids present in saliva, about one-third is inorganic ingredients and the remainder organic components such as enzymes, epithelial cell debris, and mucin.

The saliva appears to function both chemically and mechanically. Chemically, the saliva initiates the degradation of the dextrins and starches. The ptyalin of the saliva effects the hydrolysis of starches into dextrins and of dextrins into maltose, whereas the maltase of the saliva effects the hydrolysis of the maltose into two equivalents of D^+-glucose. Mechanically, the water and the mucin of the saliva aid in the complete mastication of the food as well as serving as a solvent for some of the constituents. Inasmuch as chemical bodies must be brought into intimate contact before any reaction takes place, it is obvious that the mechanical phase of digestion in the mouth is no less essential than is the chemical phase. Salivary digestion continues in the cardiac end of the stomach until the ptyalin is inactivated by the hydrochloric acid of the gastric juice. The changes which may be visualized as occurring during salivary digestion, in part at least, are (*1*) hydrolysis of starches to dextrins, (*2*) hydrolysis of dextrins to maltose, and (*3*) hydrolysis of maltose to glucose.

After the food comes in contact with the gastric juice of the stomach, the degradation of the starches and dextrins practically ceases, but the hydrolysis of the maltose apparently continues. There is some evidence to the effect that a limited amount of absorption of glucose takes place through the walls of the stomach. The three enzymes of the gastric juice, lipase, pepsin, and rennin, act primarily on the proteins, and to a limited extent on the fats.

Upon discharge from the pyloric end of the stomach, the partly digested food enters the small intestinal tract. Here it is subjected to the action of three more secretions: the bile, the intestinal juice, and the pancreatic juice. The bile acts as an emulsifying agent, promotes the solution of fats and lipids, and accelerates the activity of the lipases. It has, presumably, little or no effect on the digestion of carbohydrates.

The intestinal juice contains protein-splitting enzymes, aminopolypeptidase, dipeptidase, polynucleotidase, nucleotidase, and nucleosidase, the trypsinogen-activator enterokinase, the lipases, and the carbohydrate-splitting factors, amylase, maltase, and invertase. The amylase effects the splitting of the starches, the maltase the hydrolysis of maltose, and the invertase the inversion or hydrolysis of sucrose. The end products are the simple monosaccharides, glucose and fructose. In only a few cases has the lactose-splitting enzyme, lactase, been isolated from the intestinal juice.

The pancreatic juice contains three proteolytic enzymes, trypsin, chymotrypsin, and carboxypolypeptidase, the fat-splitting enzyme steapsin, and the carbohydrate hydrolytic factors, amylopsin, invertase, lactase, and maltase. The amylopsin converts starch to the disaccharides, and the invertase, lactase, and maltase effect, respectively, the hydrolysis of sucrose to glucose and fructose, lactose to glucose and galactose, and maltose to two equivalents of glucose.

IV. THE ANABOLISM OF THE SIMPLE SUGARS

Consideration has now been given to the carbohydrates and sugars that are utilized by the animal organism, and the digestion of these complexes in the alimentary tract. It has been observed, moreover, that in every case the end products of any utilizable carbohydrate in the digestive tract are monosaccharides such as fructose, galactose, and glucose. The anabolism of carbohydrates may be resolved then, obviously enough, into the anabolism of these simple sugars.

The utilizable monosaccharides (fructose, galactose, glucose, and mannose)[11] entering the portal circulation are deposited, to an appreciable extent, in the liver as glycogen. The liver, moreover, removes glucose from the systemic blood stream when its concentration exceeds a maximum level (about 70 to 90 mg. %) and converts it into glycogen. Thus the liver serves to regulate the sugar concentration of the blood as well as to conserve food material in the form of glycogen for subsequent use. This process of glycogen formation in the liver and, to a much smaller extent in the muscle tissue,[12] is known as glycogenesis.

The process of glycogenesis in the liver utilizes D^--fructose most readily, D^+-glucose slightly less readily, D^+-galactose still less readily, and D^+-mannose even still less readily if at all. These relative rates of conversion to glycogen are checked by feeding a large excess of the sugar and determining the amount that is excreted unchanged in the urine. In man the pentose sugars are not utilized as they are excreted unchanged in the amounts in which they are fed. Studies on the rat have definitely indicated that glycogenesis of xylose does not occur.[13] In the case of herbivorous animals, however, the pentosans are acted upon by certain bacteria in the intestinal tract and converted into organic acids and other products which the animal organism is capable of utilizing.

Aside from the three important hexoses (fructose, galactose, and glucose), glycogen may be formed in the liver from amino acids, fats, and lactic acids. Amino acids which are believed to form glycogen, when available in excess, include alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, hydroxyglutamic acid, proline, and serine.[14] In the fasting animal the glycerol, which is about 10% of the fat, is a source of both glucose and glycogen.[15] The conversion of both of the lactic acids to glycogen has been demonstrated in the white rat, although the utilization of the D^{-}-lactic acid appears to be only about one-fourth as rapid as is that of the L^{+}-lactic (or sarcolactic) acid.[16, 17]

---

[11] R. Robison, *Biochem. J.*, **26**, 2191 (1932).

[12] J. L. Bollman and F. C. Mann, *Am. J. Physiol.*, **96**, 683 (1931).

[13] M. M. Miller and H. B. Lewis, *J. Biol. Chem.*, **98**, 133, 141 (1932).

In the muscle, the glycogen is formed principally from the glucose of the blood.[12] This is in keeping with the observation that the glucose content of the arterial blood is normally slightly higher than that of venous blood. This is particularly the case immediately following a carbohydrate-rich meal. Experimental data indicate, however, that glucose is the only sugar that is utilized by muscle tissue in the formation of glycogen. The other sugars, accordingly, must pass through glucose or glycogen in the liver as an intermediary before utilization in the muscle tissues.

The synthesis of glycogen and its storage in the liver and tissues of the body are believed to be controlled by the pancreatic hormone, insulin. It is not known, however, whether the insulin promotes glycogenesis or inhibits glycogenolysis (the hydrolytic decomposition of glycogen). There is some experimental evidence which indicates that the process of glycogenesis and glycogenolysis are both controlled by the liver and not by some external secretion.[18]

Whatever the process of glycogen formation may be, it is now rather well established that fructose, glucose, and galactose all contribute to the formation of glycogen in the liver, and that only glucose forms glycogen in the muscle tissues.[19] Glucose, moreover, is the one and only product of glycogenolysis, that is, the hydrolytic product of glycogen.

V. THE CATABOLISM OF GLYCOGEN AND D^{+}-GLUCOSE

The hydrolysis of glycogen of the liver into D^{+}-glucose to meet the energy requirements of the body during periods when sugar is not being absorbed, is known as glycogenolysis. That the liver controls this delicate balance between glycogenesis and glycogenolysis and the glucose concentration of the blood stream has been clearly demonstrated. If the liver of an animal, for example, is extirpated, the concentration of the sugar in the blood rapidly diminishes and the animal soon dies. Even the glycogen that is stored in the muscle tissue is either not directly convertible into D^{+}-glucose or the mechanism is not adequate to meet such a strenuous demand.

---

[14] R. H. Wilson and H. B. Lewis, *J. Biol. Chem.*, **84**, 511 (1929). Also see H. J. Deuel, Jr., *Ann. Rev. Biochem.*, **6**, 229 (1937).

[15] L. F. Catron and H. B. Lewis, *J. Biol. Chem.*, **84**, 553 (1929).

[16] C. F. Cori and G. T. Cori, *J. Biol. Chem.*, **81**, 389 (1929). Also see H. A. Abramson, P. Eggleton, and G. P. Eggleton, *ibid.*, **75**, 763 (1927).

[17] J. H. Parnas and J. Baer, *Biochem. Z.*, **41**, 414 (1912). Also see S. Izume and H. B. Lewis, *J. Biol. Chem.*, **71**, 51 (1926–1927); C. F. Cori and G. T. Cori, *Am. J. Physiol.*, **81**, 389 (1929); C. F. Cori, *Physiol. Revs.*, **11**, 143 (1932).

[18] S. Soskin *et al.*, *Am. J. Physiol.*, **109**, 155 (1934); **110**, 4 (1934); **114**, 648 (1935–1936); **119**, 407 (1937).

[19] J. L. Bollman and F. C. Mann, *Am. J. Physiol.*, **96**, 683 (1931).

The conversion of the glycogen of the liver into D^+-glucose is effected, it is believed, by the diastatic enzyme, glycogenase. In the extirpated liver, this conversion occurs very rapidly. Cori[20] believes that the same conditions obtain *in vivo* in the absence of insulin. In the normal living organism, however, the rate of glycogenolysis is controlled either by a limited supply of the enzyme or of the substrate. Since insulin inhibits an overaccelerated rate of glycogenolysis, it is highly essential that it be supplied continuously by the pancreas. The hormone of the adrenal medulla, epinephrine, has a counter effect to that of insulin on glycogenolysis. It actually accelerates the rate of formation of D^+-glucose from the glycogen of the liver. It may be postulated that a delicate balance between these two factors may determine the rate of glycogenolysis in the liver.

The fate of muscle glycogen is different from that of liver glycogen, as was shown by Fletcher and Hopkins[21] on the frog in 1907. Twenty years later, Simpson and Macleod[22] carried out a similar series of studies on the rabbit. In these investigations they used rabbits on a standard diet, killed them at various degrees of exhaustion, froze the liver and muscle tissues in liquid air, ground these tissues to a powder, and then analyzed for glycogen, reducing sugar, and lactic acid. As a result of their experiments they concluded that a decrease of glycogen in the liver is accompanied by an increase in reducing sugar but no change in the concentration of lactic acid, whereas a decrease of glycogen in muscle tissue is accompanied by an increase in lactic acid but no change in the concentration of reducing sugar. These studies seem to indicate that either glycogenolysis of muscle glycogen yields (*a*) lactic acid directly, or (*b*) D^+-glucose as a momentary intermediate which is instantly converted to lactic acid.

Inasmuch, then, as all carbohydrate material which is utilized by the animal organism is converted to D^+-glucose, either directly or through glycogenesis and glycogenolysis, and inasmuch as lactic acid formed by glycogenolysis of muscle tissue is also a product of the catabolism of D^+-glucose, consideration may now be given to the catabolism of glycogen and D^+-glucose.

If D^+-glucose is an intermediate in the catabolism of the glycogen of muscle tissue, it is apparent from data available that the conversion of D^+-glucose to lactic acid in the presence of muscle extract should occur much more rapidly than does the conversion of glycogen to lactic acid under comparable experimental conditions. Tests *in vitro*, however, indicate that this is not the case. Fructose and glucose, in the presence of muscle extract, yield lactic acid much less readily than does glycogen.

When muscle extract is heated for some time at 38° C., it loses its capacity to convert glycogen to lactic acid but retains its activity for the conversion of glycogen to glucose. This partially inactivated extract, however, does retain its ability to convert hexosediphosphoric acid into lactic acid. This suggests that the effect of the heat treatment might be the destruction of the phosphoric acid esterification enzyme. This view has been confirmed, in part at least, by Meyerhof[23] who found that the addition of hexokinase (an extract from yeast) to muscle extract greatly accelerates the conversion of D^+-glucose to lactic acid. Hexokinase appears to function as an esterification catalyst. In its presence, for example, the phosphoric acid ester of D^--fructose is formed about twice as rapidly as is that of D^+-glucose. These observations along with others led to the conviction that a phosphoric acid ester is an intermediate in the production of lactic acid from glycogen in muscle tissue.

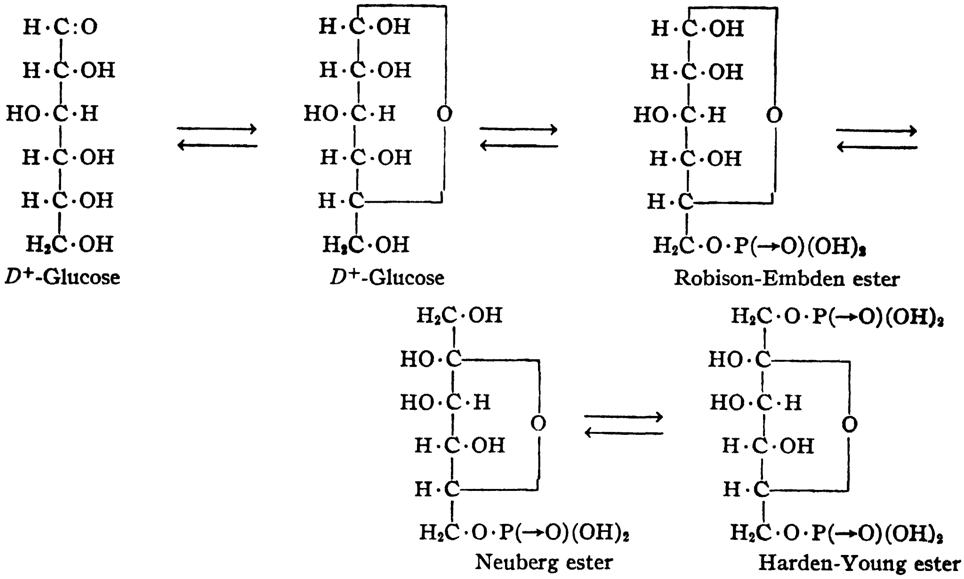
---

[20] C. F. Cori, *Physiol. Revs.*, **11**, 143 (1931).

[21] W. M. Fletcher and F. G. Hopkins, *J. Physiol.*, **35**, 247 (1907).

[22] W. W. Simpson and J. J. R. Macleod, *J. Physiol.*, **64**, 255 (1927).

As early as 1911, Harden and Young[24] observed that phosphate accelerates the fermentation of sugars by yeast, and succeeded in the isolation of a hexosediphosphoric acid from the fermentation products. This hexosediphosphoric acid was isolated and identified by other workers also. Two other esters, moreover, have been reported and are believed to be components of a phosphoric acid-hexose equilibrium system as shown in formula XII.



Formula XII.—Hexose-phosphoric acid equilibrium.[25–27]

The existence of the monophosphoric ester of the hexoses was suspected by Harden and Robison[27] in 1914, at which time Embden *et al.*,[28] isolated from muscle press juice an ester identical with that of Harden and Young. Eight years later (1922) Robison[29] succeeded in isolating a hexosemonophosphoric acid, which was not identical with that described by Neuberg.[26] Embden and Zimmermann,[30] in 1924, isolated a hexosemonophosphoric acid from muscle extract. This ester was finally shown to be identical with that of Robison in that both esters consisted of an equilibrium mixture of the glucosemonophosphoric acid (70%) and the fructosemonophosphoric acid (30%). Subsequent workers have isolated trehalosemonophosphoric acid,[31] from the fermentation products of either fructose or glucose with dry yeast, and mannosemonophosphoric acid.[32]

---

[23] O. Meyerhof, *Die chemischen Vorgänge im Muskel.* Springer, Berlin, 1930.

[24] A. Harden and W. J. Young, *Biochem. Z.*, **32,** 173 (1911).

[25] E. J. King, R. R. McLaughlin, and W. T. J. Morgan, *Biochem. J.*, **25,** 310 (1931); R. Robison and E. J. King, *ibid.*, **25,** 323 (1931). Also see P. A. Levene and A. L. Raymond, *J. Biol. Chem.*, **89,** 479 (1930); **91,** 751 (1931).

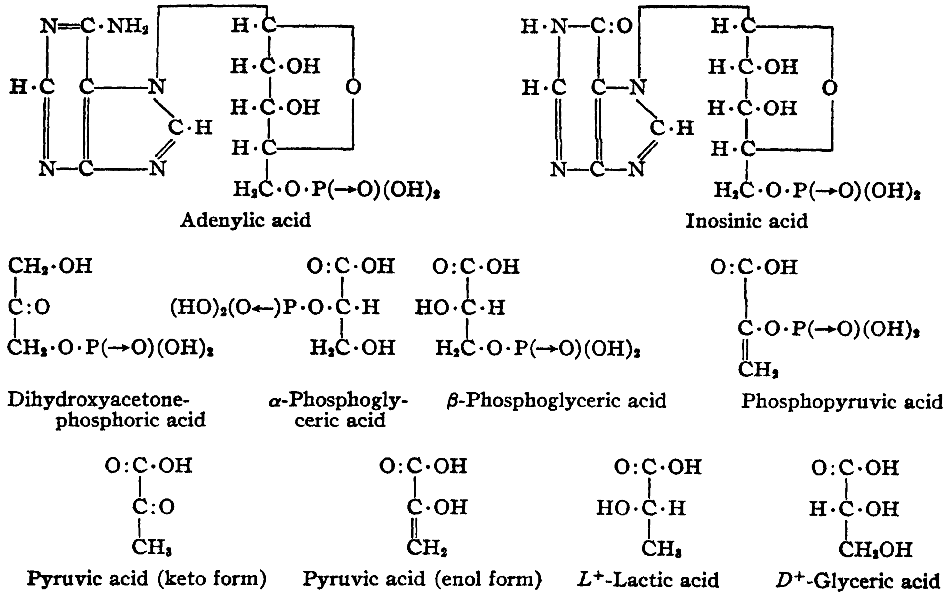
[26] C. Neuberg, *Biochem. Z.*, **88,** 432 (1918).

[27] A. Harden and R. Robison, *Proc. Chem. Soc.*, **30,** 16 (1914).

[28] G. Embden *et al.*, *Z. physiol. Chem.*, **93,** 124 (1914).

[29] R. Robison, *Biochem. J.*, **16,** 809 (1922).

Other catabolic products which have been reported are shown in formula XIII.



Adenylic acid

Inosinic acid

Dihydroxyacetone-phosphoric acid

α-Phosphoglyceric acid

β-Phosphoglyceric acid

Phosphopyruvic acid

Pyruvic acid (keto form)

Pyruvic acid (enol form)

L^{+}-Lactic acid

D^{+}-Glyceric acid

Formula XIII.—Structure of some catabolic products.

As a consequence of the isolation of the hexosephosphoric esters and the other catabolic intermediates, mechanisms of the *in vivo* degradation of both glycogen and glucose to lactic acid have been formulated. The concept of Parnas,[33] in diagrammatic form, may be represented as shown in Fig. 66.

According to this proposed mechanism (Fig. 66), the phosphates under the influence of an enzyme (ketokinase or similar activator) combine directly with the glycogen (*a*) either to give a momentary addition complex which undergoes cleavage to fructosediphosphate, or (*b*) to effect direct cleavage into units of fructosediphosphate. According to Shaffer and Ronzoni,[34] epinephrine increases the conversion of muscle glycogen into hexosephosphates and the insulin in turn accelerates the further disintegration of these complexes into their ultimate end products—carbon dioxide and water.

---

[30] G. Embden and M. Zimmermann, *Z. physiol. Chem.*, **141**, 225 (1924).

[31] R. Robison and W. T. J. Morgan, *Biochem. J.*, **22**, 1277 (1928).

[32] R. Robison, *Biochem. J.*, **26**, 2191 (1932).

[33] J. H. Parnas, *Klin. Wochschr.*, **14**, 1017 (1935).

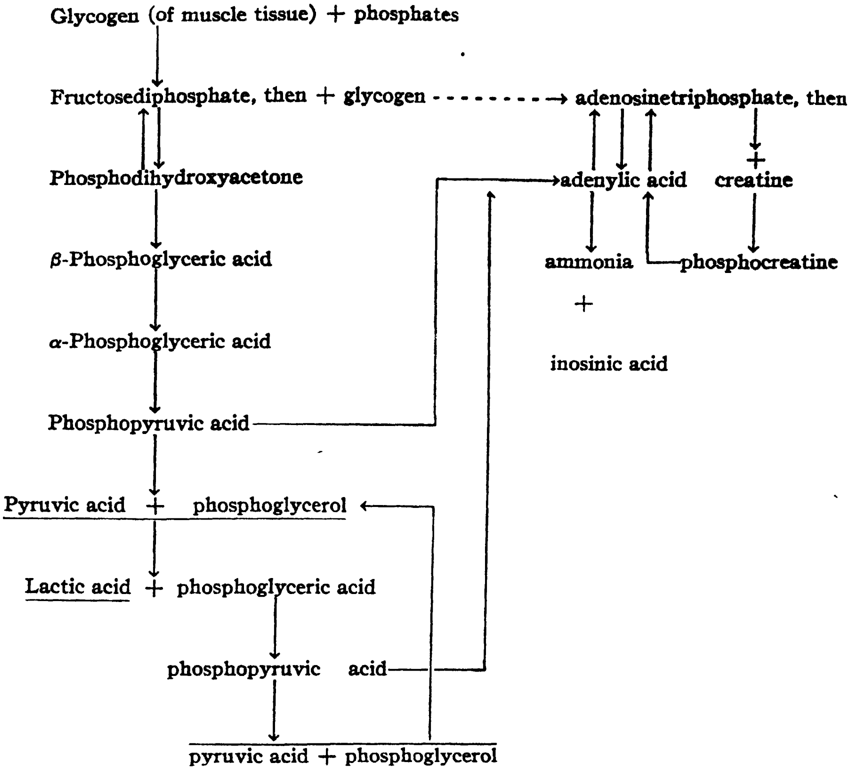

Glycogen (of muscle tissue) + phosphates
Fructosediphosphate, then + glycogen
adenosinetriphosphate, then
Phosphodihydroxyacetone
adenylic acid
+
creatine
β-Phosphoglyceric acid
ammonia
phosphocreatine
+
inosinic acid
α-Phosphoglyceric acid
Phosphopyruvic acid
Pyruvic acid + phosphoglycerol
Lactic acid + phosphoglyceric acid
phosphopyruvic acid
pyruvic acid + phosphoglycerol

Fig. 66.—Modified Parnas concept of catabolism of glycogen.

According to the Meyerhof and Kiessling concept, the glycogen of muscle tissue is first hydrolyzed to yield D^+-glucose units which are immediately attacked by the adenylpyrophosphates to give hexosediphosphates and adenylic acid, as represented in Fig. 67. [35–37]

Since it is known that glycogenase converts the glycogen of the liver into D^+-glucose units, this representation of Meyerhof and Kiessling is appropriate for the disintegration of both the glycogen of liver and muscle tissue. It should be noted that, according to both of these concepts (Figs. 66 and 67) lactic acid appears as an important metabolic intermediate. What happens to lactic acid, then, is of immediate interest.

---

[34] P. A. Shaffer and E. Ronzoni, *Ann. Rev. Biochem.*, **1**, 247 (1932).
[35] O. Meyerhof and W. Kiessling, *Biochem. Z.*, **283**, 83 (1936).
[36] P. A. Levene and A. L. Raymond, *J. Biol. Chem.*, **80**, 633 (1928).
[37] W. T. J. Morgan and R. Robison, *Biochem. J.*, **22**, 1270 (1928).

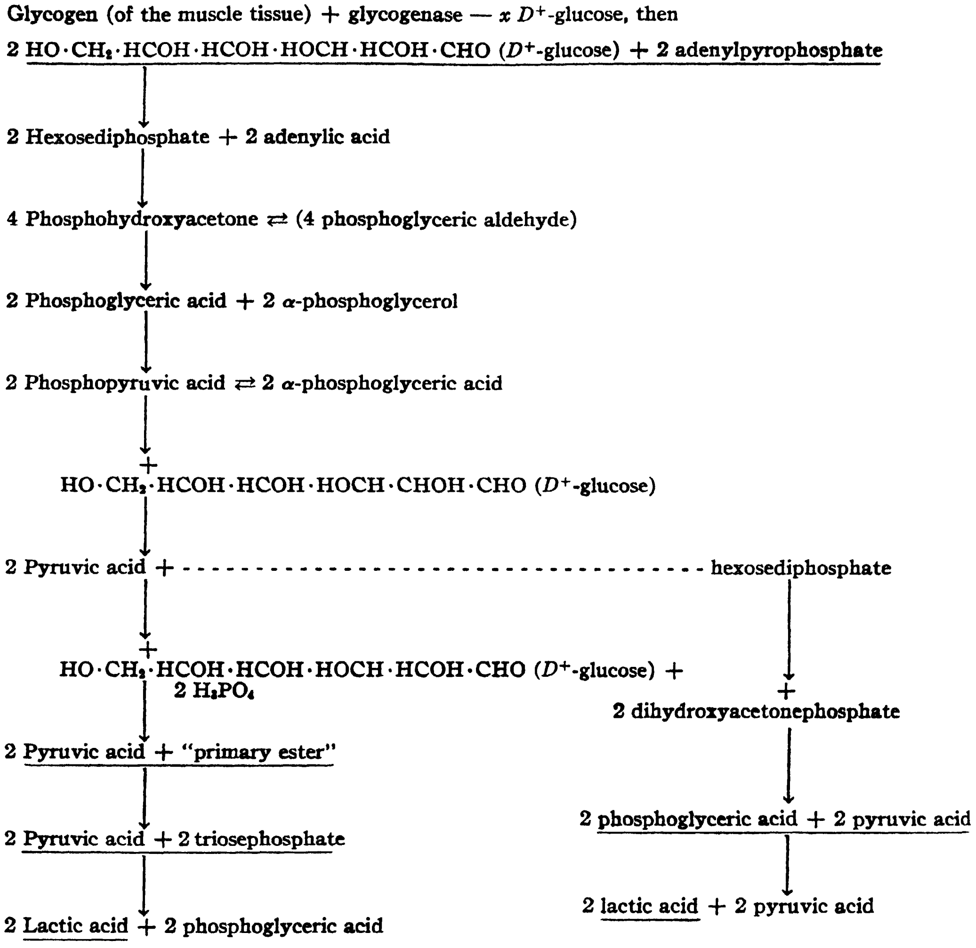
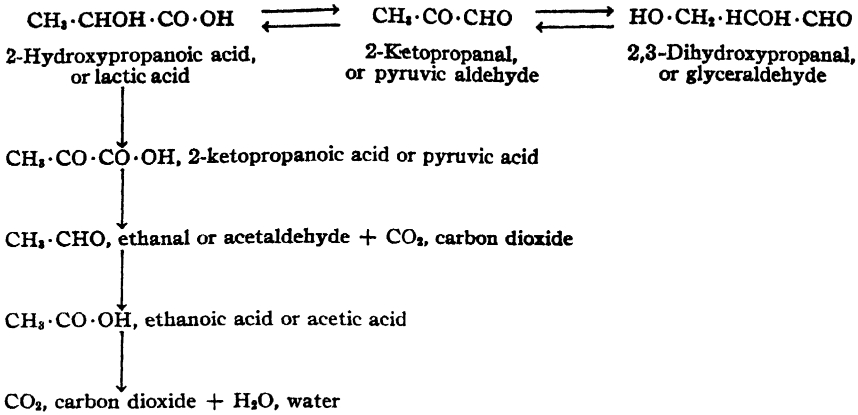

Glycogen (of the muscle tissue) + glycogenase — x D^+-glucose, then
2 HO·CH$_2$·HCOH·HCOH·HOCH·HCOH·CHO (D^+-glucose) + 2 adenylpyrophosphate
2 Hexosediphosphate + 2 adenylic acid
4 Phosphohydroxyacetone ⇄ (4 phosphoglyceric aldehyde)
2 Phosphoglyceric acid + 2 α-phosphoglycerol
2 Phosphopyruvic acid ⇄ 2 α-phosphoglyceric acid
+
HO·CH$_2$·HCOH·HCOH·HOCH·CHOH·CHO (D^+-glucose)
2 Pyruvic acid + hexosediphosphate
+
HO·CH$_2$·HCOH·HCOH·HOCH·HCOH·CHO (D^+-glucose) +
2 H$_3$PO$_4$
+
2 dihydroxyacetonephosphate
2 Pyruvic acid + "primary ester"
2 phosphoglyceric acid + 2 pyruvic acid
2 Pyruvic acid + 2 triosephosphate
2 lactic acid + 2 pyruvic acid
2 Lactic acid + 2 phosphoglyceric acid

Fig. 67.—Meyerhof-Kiessling concept of glycogen catabolism.

The lactic acid formed during the catabolism of glycogen and D^+-glucose may be (*1*) oxidized through intermediates to carbon dioxide and water, (*2*) reconverted to glycogen in the muscle tissue, or (*3*) diffused into the blood and either reconverted to glycogen by the liver or excreted by the kidneys. The oxidation of the lactic acid resulting from glycogenolysis to carbon dioxide and water to supply energy is indicated in Fig. 68.



$CH_3\cdot CHOH\cdot CO\cdot OH \rightleftharpoons CH_3\cdot CO\cdot CHO \rightleftharpoons HO\cdot CH_2\cdot HCOH\cdot CHO$

2-Hydroxypropanoic acid, or lactic acid; 2-Ketopropanal, or pyruvic aldehyde; 2,3-Dihydroxypropanal, or glyceraldehyde

$\downarrow$

$CH_3\cdot CO\cdot CO\cdot OH$, 2-ketopropanoic acid or pyruvic acid

$\downarrow$

$CH_3\cdot CHO$, ethanal or acetaldehyde + CO_2, carbon dioxide

$\downarrow$

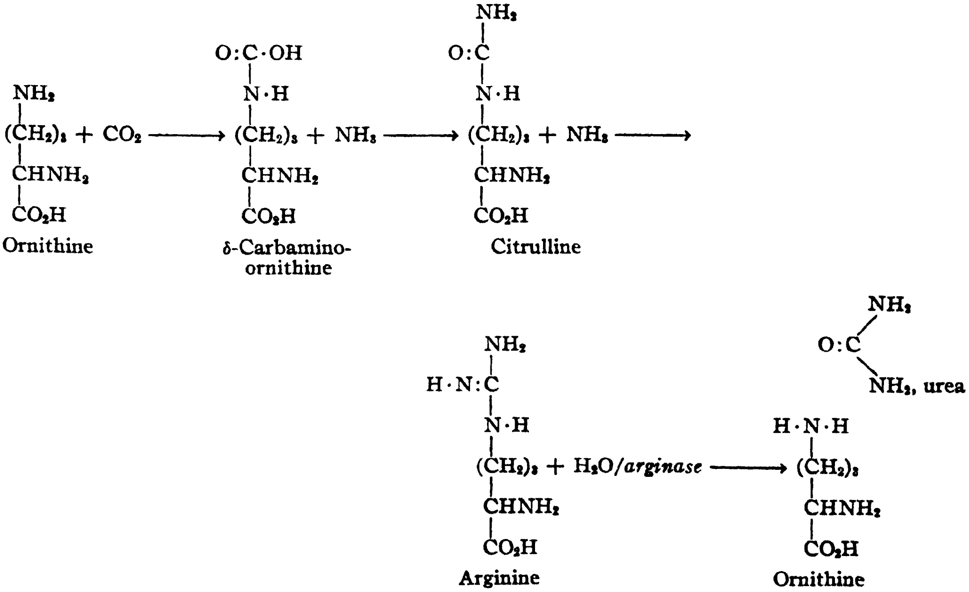
$CH_3\cdot CO\cdot OH$, ethanoic acid or acetic acid

$\downarrow$

CO_2, carbon dioxide + H_2O, water

Fig. 68.—Oxidation of lactic acid to end products.

In this series of oxidations (Fig. 68), the end products are carbon dioxide and water. The water is expelled through the lungs, skin, or kidneys, whereas the carbon dioxide may be expelled through the lungs or converted into urea and expelled through the kidneys. The mechanism postulated for the disposal of carbon dioxide through the formation of urea is shown in Fig. 69.



$$\begin{array}{l} NH_2 \\ \mid \\ (CH_2)_3 \\ \mid \\ CHNH_2 \\ \mid \\ CO_2H \end{array} + CO_2 \longrightarrow \begin{array}{l} O{:}C\cdot OH \\ \mid \\ N\cdot H \\ \mid \\ (CH_2)_3 \\ \mid \\ CHNH_2 \\ \mid \\ CO_2H \end{array} + NH_3 \longrightarrow \begin{array}{l} NH_2 \\ \mid \\ O{:}C \\ \mid \\ N\cdot H \\ \mid \\ (CH_2)_3 \\ \mid \\ CHNH_2 \\ \mid \\ CO_2H \end{array} + NH_3 \longrightarrow$$

Ornithine; δ-Carbamino-ornithine; Citrulline

$$\begin{array}{l} NH_2 \\ \mid \\ H\cdot N{:}C \\ \mid \\ N\cdot H \\ \mid \\ (CH_2)_3 \\ \mid \\ CHNH_2 \\ \mid \\ CO_2H \end{array} + H_2O/\mathit{arginase} \longrightarrow O{:}C\begin{array}{l} \diagup NH_2 \\ \diagdown NH_2 \end{array}\text{, urea} \quad \begin{array}{l} H\cdot N\cdot H \\ \mid \\ (CH_2)_3 \\ \mid \\ CHNH_2 \\ \mid \\ CO_2H \end{array}$$

Arginine; Ornithine

Fig. 69.—Elimination of carbon dioxide as urea.

The conversion of lactic acid into glycogen in muscle tissue has been demonstrated.[38] In isolated frog muscle, for example, Meyerhof[39] showed that about four-fifths of the lactic acid produced during the contraction is resynthesized to glycogen during the rest period. This is considered to be an important factor, during the recovery period, in the restoration of the muscle to its original status. Results by Boyland on isolated rabbit and beef muscle confirm the observations of Meyerhof, whereas somewhat contradictory results have been reported by Sacks and Sacks[39] on studies of changes in the gastrocnemius of the living rabbit.

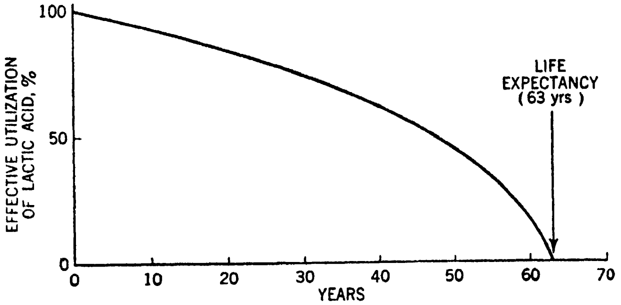


Fig. 70.—Life expectancy of an average adult.

The fate of the lactic acid in catabolism is intimately tied up with a very general problem—why do we grow old?[40] It has been postulated that the inability of youth to maintain their vigor indefinitely is due to a decrease in the effective utilization of lactic acid, as indicated by Fig. 70. Statistical studies indicate, for example, that life expectancy would be about 550 years instead of 63 if the death rate for all human beings were reduced to the rate for ten-year-olds. Can science maintain the adolescent equilibrium between the processes of anabolism and catabolism, and thus appreciably lengthen the span of life? Can glycogen be synthesized as efficiently in the muscle tissues from lactic acid at the age of 70 as it can be at the age of 10 years?

During a period of rest the blood contains lactic acid in amounts varying between 5 and 20 mg. per 100 ml. of blood.[41] With exercise there is an increase in the lactic acid concentration of the blood which is directly proportional to the amount of exercise but inversely proportional to the amount of oxygen available.[42] Some data are given in Table 192.

---

[38] J. H. Parnas and J. Baer, *Biochem. Z.*, **41**, 414 (1912). Also see S. Izume and H. B. Lewis, *J Biol. Chem.*, **71**, 51 (1926-1927); C. F. Cori and G. T. Cori, *Am. J. Physiol.*, **81**, 389 (1929); C. F. Cori, *Physiol. Revs.*, **11**, 143 (1932).

[39] O. Meyerhof, *Arch. ges. Physiol.*, **185**, 11 (1920). *Cf.* Hill, *Physiol. Revs.*, **2**, 329 (1922). Also E. Boyland, *Biochem. Z.*, **237**, 418 (1931); J. Sacks and W. C. Sacks, *Am. J. Physiol.*, **112**, 565 (1935)

[40] Ed. F. Degering *et al.*, *An Outline of Organic Chemistry.* Barnes and Noble, New York, 1942, p. 159. *Fundamental Organic Chemistry.* Swift & Co., Cincinnati, 1942, p. 344. *An Outline of Organic Nitrogen Compounds.* Purdue Univ., Lafayette, 1942, pp. 95, 108, 112. *An Outline of the Chemistry of the Carbohydrates.* Purdue Univ., Lafayette, 1941, p. 138.

[41] A. V. Bock, D. B. Dill, and H. T. Edwards, *J. Clin. Investigation*, **11**, 775 (1932).

[42] A. V. Hill, C. N. H. Long, and H. Lupton, *Proc. Roy. Soc. London*, **B96**, 438 (1924); **B97**, 84, 155 (1924).

As the concentration of the lactic acid in the blood is gradually increased, the conversion to glycogen as well as the rate of elimination through the kidneys is increased. When these two opposing tendencies are in balance the condition is described as one of steady state. As the exercise becomes increasingly severe, the lactic acid tends to accumulate in the muscle tissue because the supply of oxygen cannot be increased beyond a certain maximum. During the subsequent rest period, however, the lactic acid will be removed either by conversion to glycogen and/or through elimination, and the muscle tissue will be restored to normal. Barr and Himwich[43] have shown, however, that in cases of violent exercise the build-up of lactic acid in the blood may continue for an interval after the exercise is suddenly terminated.

TABLE 192

SHOWING LACTIC ACID CONCENTRATION OF BLOOD

Initial concentration of lactic acid, mg./100 ml. blood	State of subject	Final concentration of lactic acid, mg./100 ml. of blood
5–20	At rest	5–20
20.9	Walking, 3.5 mi./hr.	36.6
21.4	Walking, 4.1 mi./hr.	58.9

The fate of the lactic acid of the blood has been studied by Himwich, Koskoff, and Nahum,[44] who studied the lactic acid concentration of both arterial and venous blood to and from different tissues and organs of the body. In general, they found that blood leaving muscle tissue contains *more* lactic acid than the blood entering the tissue, whereas the blood leaving the liver contains *less* lactic acid than that entering the liver. In the case of a dog that had been exercised for 5 min., these workers found the milligram concentration of lactic acid per 100 ml. of blood to be: 65 for the hepatic artery, 42 for the portal vein, and 24 for the hepatic vein. Bott and Wilson,[45] moreover, have shown that the decrease in the lactic acid concentration of the blood after passing through the liver does not result in a corresponding increase in the lactic acid content of the liver tissue. A number of experiments have been carried out to demonstrate that sodium lactate and lactic acid are assimilated and converted to glycogen in the liver.[46]

The cycle that lactic acid passes through in the animal organism may be shown as indicated on page 806, according to the work of Himwich[47] and of Cori and Cori.[48]

---

[43] D. P. Barr and H. E. Himwich, *Am. J. Physiol.*, **106**, 689 (1933).

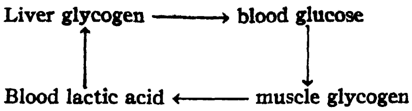
[44] H. E. Himwich, Y. D. Koskoff, and L. H. Nahum, *J. Biol. Chem.*, **85**, 571 (1930).

[45] P. A. Bott and D. W. Wilson, *J. Biol. Chem.*, **109**, 463 (1935).

[46] J. H. Parnas and J. Baer, *Biochem. Z.*, **41**, 414 (1912). Also see S. Izume and H. B. Lewis, *J. Biol. Chem.*, **71**, 51 (1926–1927); C. F. Cori and G. T. Cori, *Am. J. Physiol.*, **81**, 389 (1929); C. F. Cori, *Physiol. Revs.*, **11**, 143 (1932).

[47] H. E. Himwich *et al.*, *J. Biol. Chem.*, **85**, 571 (1930). Also see C. F. Cori and G. T. Cori, *Am. J. Physiol.*, **81**, 389 (1929).

[48] C. F. Cori and G. T. Cori, *J. Biol. Chem.*, **81**, 389 (1929). Also see H. A. Abramson, G. P. Eggleton, and P. Eggleton, *ibid.*, **75**, 763 (1927).



Fermentation is another way in which the hexose sugars may be utilized, to a limited extent at least, in the animal organism. The mechanism proposed by Meyerhof *et al.*,[49] is shown in Fig. 71.

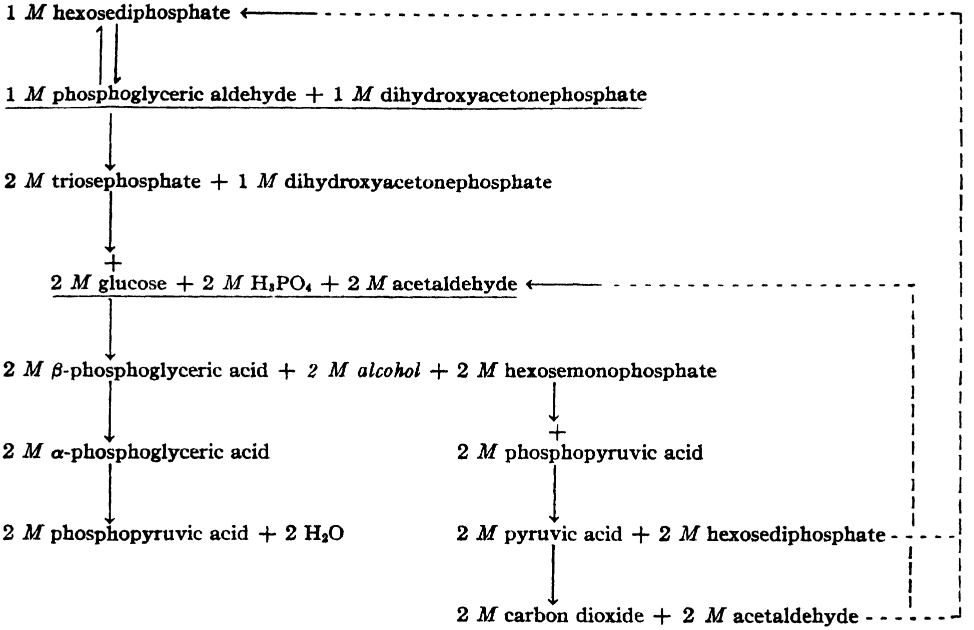

1 M hexosediphosphate
1 M phosphoglyceric aldehyde + 1 M dihydroxyacetonephosphate
2 M triosephosphate + 1 M dihydroxyacetonephosphate
+
2 M glucose + 2 M H₃PO₄ + 2 M acetaldehyde
2 M β-phosphoglyceric acid + 2 M alcohol + 2 M hexosemonophosphate
2 M α-phosphoglyceric acid
+
2 M phosphopyruvic acid
2 M phosphopyruvic acid + 2 H₂O
2 M pyruvic acid + 2 M hexosediphosphate
2 M carbon dioxide + 2 M acetaldehyde

Fig. 71.—Mechanism of fermentation of hexoses.

Rather extensive studies[50] have shown that both D^{+}-glucose and lactic acid are utilized in the respiratory metabolism of the brain, as evidenced by studies on dogs in which the blood going from and to the brain was analyzed for these components.

VI. THE INTERRELATIONSHIP OF CARBOHYDRATES, FATS, AND PROTEINS

Because of the capacity of the animal organism to convert either carbohydrates, fats, or proteins into either one of the other two, the story of the utilization of carbohydrates as foods is not complete without consideration of this possibility. Diets and feeding experiments have demonstrated rather conclusively that excess carbohydrate, particularly in a fat-deficient diet, may be converted to fat and stored as such in the adipose tissue.

---

[49] O. Meyerhof and W. Kiessling, *Biochem. Z.*, **281**, 249 (1935); O. Meyerhof, W. Kiessling, and W. Schultz, *ibid.*, 292, 25 (1937).

[50] H. E. Himwich and L. H. Nahum, *Am. J. Physiol.*, **90**, 389 (1929); **101**, 446 (1932). Also see I. H. Page, *Chemistry of the Brain*. Charles C. Thomas, Springfield, Ill., 1937.

Attention has already been called to the fact that acetaldehyde may be a metabolic intermediate (*cf.* Fig. 68). According to the postulate of Smedley and Lubrzynska,[51] this intermediate may undergo an aldol type condensation in accordance with the following equations to yield an acid:

$$\mathrm{CH_3{\cdot}CHO + CH_3{\cdot}CO{\cdot}CO{\cdot}H \longrightarrow CH_3{\cdot}CH(OH){\cdot}CH_2{\cdot}CO{\cdot}CO{\cdot}H}, \text{ then}$$

$$\xrightarrow{+\ {}^1/_2\mathrm{O_2}\ \text{(biochemical oxidation)}} \mathrm{CH_3{\cdot}CH(OH){\cdot}CH_2{\cdot}CO{\cdot}CO{\cdot}OH}, \text{ then}$$

$$\xrightarrow{-\ \mathrm{H_2O}\ \text{(biochemical dehydration)}} \mathrm{CH_3{\cdot}HC{:}CH{\cdot}CO{\cdot}CO{\cdot}OH}, \text{ then}$$

$$\xrightarrow{-\ \mathrm{CO_2}\ \text{(biochemical decarboxylation)}} \mathrm{CH_3{\cdot}HC{:}CH{\cdot}CO{\cdot}H}, \text{ then}$$

$$\xrightarrow{+\ \mathrm{H_2}\ \text{(biochemical hydrogenation)}} \mathrm{CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}CO{\cdot}H}, \text{ butanal.}$$

By this proposed mechanism, two carbon atoms have been added to the acetaldehyde unit as a consequence of biochemical condensation, oxidation, dehydration, decarboxylation, and hydrogenation. The butanal in turn may undergo a similar condensation with another equivalent of pyruvic aldehyde, as indicated by:

$$\mathrm{CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}CHO + CH_3{\cdot}CO{\cdot}CO{\cdot}H \longrightarrow CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}CH(OH){\cdot}CH_2{\cdot}CO{\cdot}CO{\cdot}H}, \text{ then}$$

$$\xrightarrow{+\ {}^1/_2\mathrm{O_2},\ \text{biochemical oxidation}} \mathrm{CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}CH(OH){\cdot}CH_2{\cdot}CO{\cdot}CO{\cdot}OH}, \text{ then}$$

$$\xrightarrow{-\ \mathrm{H_2O},\ \text{biochemical dehydration}} \mathrm{CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}HC{:}CH{\cdot}CO{\cdot}CO{\cdot}OH}, \text{ then}$$

$$\xrightarrow{-\ \mathrm{CO_2},\ \text{biochemical decarboxylation}} \mathrm{CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}HC{:}CH{\cdot}CO{\cdot}H}, \text{ then}$$

$$\xrightarrow{+\ \mathrm{H_2O},\ \text{biochemical hydrogenation}} \mathrm{CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CH_2{\cdot}CHO}, \text{ hexanal.}$$

The hexanal in turn condenses with an equivalent of pyruvic aldehyde to yield octanal. The octanal in turn yields decanal, the decanal yields dodecanal, and so on until the building-up process is discontinued by biochemical oxidation of the aldehyde to the corresponding acid, in accordance with the equation

$$\mathrm{CH_3{\cdot}(CH_2)_n{\cdot}CHO} + {}^1/_2\mathrm{O_2}, \text{ biochemical oxidation} \longrightarrow \mathrm{CH_3{\cdot}(CH_2)_n{\cdot}CO{\cdot}OH}, \text{ an alkanoic acid}$$

The lactic acid-pyruvic aldehyde-glyceraldehyde equilibrium is shown in Fig. 68. Presumably, any one of these degradation products of D^{+}-glucose may undergo biochemical oxidation and/or reduction to yield glycerol, as indicated by

$$\mathrm{CH_3{\cdot}CHOH{\cdot}CO{\cdot}OH + 2H} \xrightarrow{\text{biochemical reduction}} \mathrm{HO{\cdot}CH_2{\cdot}CHOH{\cdot}CH_2{\cdot}OH}, \text{ glycerol, or}$$

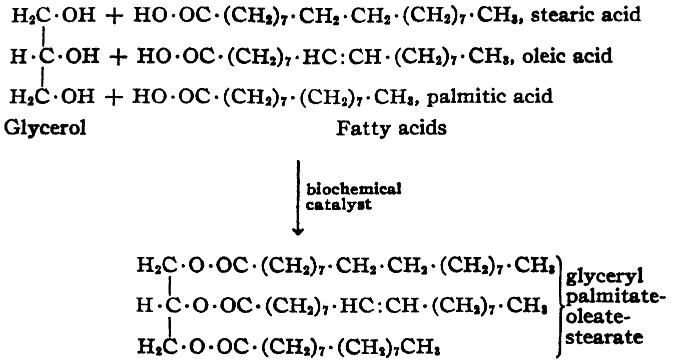
$$\mathrm{CH_3{\cdot}CO{\cdot}CO{\cdot}H + 4H + O}, \xrightarrow{\text{biochemical oxidation-reduction}} \mathrm{HO{\cdot}CH_2{\cdot}CHOH{\cdot}CH_2{\cdot}OH}, \text{ and}$$

$$\mathrm{HO{\cdot}CH_2{\cdot}CHOH{\cdot}CO{\cdot}H + 2H}, \xrightarrow{\text{biochemical reduction}} \mathrm{HO{\cdot}CH_2{\cdot}CHOH{\cdot}CH_2{\cdot}OH}, \text{ glycerol.}$$

---

[51] I Smedley and E. Lubrzynska, *Biochem. J.*, **7**, 364, 375 (1913).

The glycerol obtained from one of these reactions may condense in turn with the fatty acid, obtained through the Smedley and Lubrzynska condensation, to give a fat in accordance with the equation



$$\begin{array}{ll}
H_2C\cdot OH + HO\cdot OC\cdot (CH_2)_7\cdot CH_2\cdot CH_2\cdot (CH_2)_7\cdot CH_3, & \text{stearic acid}\\
\quad | & \\
H\cdot C\cdot OH + HO\cdot OC\cdot (CH_2)_7\cdot HC{:}CH\cdot (CH_2)_7\cdot CH_3, & \text{oleic acid}\\
\quad | & \\
H_2C\cdot OH + HO\cdot OC\cdot (CH_2)_7\cdot (CH_2)_7\cdot CH_3, & \text{palmitic acid}\\
\text{Glycerol} \qquad\qquad\qquad \text{Fatty acids} &
\end{array}$$

$$\Big\downarrow \text{biochemical catalyst}$$

$$\left.\begin{array}{l}
H_2C\cdot O\cdot OC\cdot (CH_2)_7\cdot CH_2\cdot CH_2\cdot (CH_2)_7\cdot CH_3\\
\quad | \\
H\cdot C\cdot O\cdot OC\cdot (CH_2)_7\cdot HC{:}CH\cdot (CH_2)_7\cdot CH_3\\
\quad | \\
H_2C\cdot O\cdot OC\cdot (CH_2)_7\cdot (CH_2)_7CH_3
\end{array}\right\}\begin{array}{l}\text{glyceryl}\\ \text{palmitate-}\\ \text{oleate-}\\ \text{stearate}\end{array}$$

Oleic, palmitic, and stearic acids have been used in this example because they are the three fatty acids which are produced most abundantly in nature. In any given fat there may be any possible combination of fatty acid residue, that is, three of a given acid, two of a given acid and one of another, or three of three different acids.

Attention has been given to the probability of lactic acid yielding such intermediates as glyceraldehyde, glycerol, pyruvic aldehyde, pyruvic acid, and acetaldehyde. It has been shown, furthermore, that normal catabolic products of both D^+-glucose and glycogen are lactic acid. It should now be suggested that proteins, too, are a potential source of lactic acid.

Alanine ($CH_3\cdot CHNH_2\cdot CO\cdot OH$), according to Bergmann *et al.*,[52] is every fourth building unit in the silk fibroin molecule. The animal organism, moreover, is capable of synthesizing alanine. Alanine, in turn, may be converted to lactic acid through hydrolytic deamination, to pyruvic aldehyde through direct deamination, or to pyruvic acid through oxidative deamination (Fig. 72).

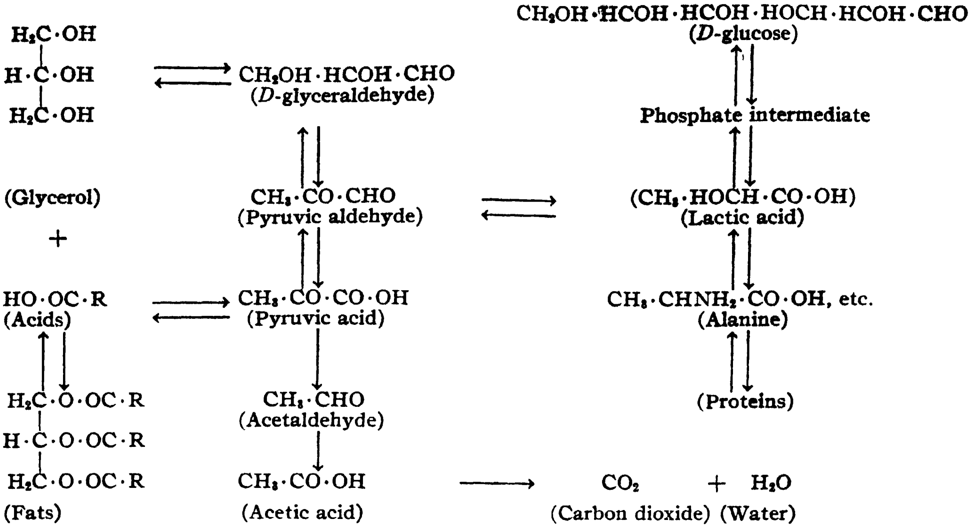
$$CH_3\cdot CHNH_2\cdot CO_2H \left\{\begin{array}{l}
+ \text{ hydrolytic deamination} \longrightarrow CH_3\cdot CHOH\cdot CO_2H,\ \text{lactic acid or}\\
+ \text{ reductive deamination} \longrightarrow CH_3\cdot CH_2\cdot CO_2H,\ \text{propionic acid, or}\\
+ \text{ oxidative deamination} \longrightarrow CH_3\cdot CO\cdot CO_2H,\ \text{pyruvic acid, and}\\
+ \text{ direct deamination} \longrightarrow CH_3\cdot CO\cdot CO\cdot H,\ \text{pyruvic aldehyde}
\end{array}\right.$$

Fig. 72.—Deamination of alanine.

Since glycerol and the fatty acids are supplied by the hydrolysis of fats, and since glycerol and lactic acid are interconvertible, and since lactic acid may be produced from either the oxidation of D^+-glucose or the glycogenolysis of glycogen or the deamination of alanine, it must be apparent that an interrelationship of the type shown in formula XIV must exist between carbohydrates, fats, and proteins.

---

[52] M. Bergmann *et al.*, *J. Biol. Chem.*, **109**, 317 (1935); **110**, 471 (1935); **113**, 341 (1936); **122**, 569, 577 (1938); *Chem. Revs.*, **22**, 423 (1939).



Formula XIV.—Interrelationship between carbohydrates, fats, and proteins.

It seems apparent, then (*cf.* formula XIV), that the animal organism can maintain, within certain limits at least, a rather delicate balance between the carbohydrate, fat, and protein requirements irrespective of the ingested food. This is indeed fortunate in view of the fact that certain regions of the world are forced to exist almost wholly on a protein diet, whereas in other localities a carbohydrate or fat diet is predominant.

Much progress has been made relative to an understanding of the food requirements of the animal organism and the manner in which this food is transformed into energy and tissue, but the biochemist and the food expert are, like Newton, merely playing on the sandy beaches with the great oceans of truth yet unexplored.

Selected Bibliography

Degering, Ed. F., *et al.*, *An Outline of the Chemistry of the Carbohydrates.* Purdue Univ., Lafayette, 1941.

Degering, Ed. F., *et al.*, *An Outline of Organic Chemistry.* Barnes and Noble, New York, 1942.

Degering, Ed. F., *et al.*, *An Outline of Organic Nitrogen Compounds.* Purdue Univ., Lafayette, 1942.

Degering, Ed. F., *et al.*, *Fundamental Organic Chemistry.* Swift and Co., Cincinnati, 1942.

Matthews, A. P., *Physiological Chemistry.* 5th ed., Wm. Wood, Baltimore, 1930.

Meyerhof, O., *Die chemischen Vorgänge im Muskel.* Springer, Berlin, 1930.

Page, I. H., *Chemistry of the Brain.* Charles C Thomas, Springfield, Ill., 1937.

Rettger, L. F., *et al.*, *Lactobacillus Acidophilus and Its Therapeutic Applications.* Yale Univ. Press, New Haven, 1935.

Sherman, H. C., *Chemistry of Food and Nutrition.* Macmillan, New York, 1941.

Chapter XXI

CONFECTIONERY AND CACAO PRODUCTS

MARTIN SCHOEN

Brooklyn, New York

> *"And the Children of Israel called the name thereof Manna, and it was white like coriander seed and the taste of it was like wafers made with honey."* Exodus XVI : 31.

I. HISTORICAL BACKGROUND

The origin of the art of making candy is hidden in the darkness of ancient times. Since honey, probably the original sweetening agent, is mentioned in the Bible and Egyptian papyri it is logical to assume that our present-day use of sugar in the making of candy is the evolution of a very old science. The early Greeks and Romans were accustomed to finish their elaborate feasts with some sort of sweet dish, usually a mixture of fruit and honey or flour paste and honey. Nasiri-Khosran, famous traveler and historian of the 11th century, in describing the great feast of Ramazan mentions that the Sultan's table was decorated with marzipan sweetmeats made of sweet almonds and powdered sugar, and moulded into shapes of trees and statues. Early explorers of the New World, in the 16th century, found that the Indians in Mexico had several confections; one in particular was found to be very nourishing and sustaining. This confection was obtained from cacao seeds which were ground to a paste with various spices and dried fruits.

Candy, at first, was sold by the apothecaries and the spice stores. This was to be expected since the early use of sweets was to disguise the bitter taste of many medicines. Later, the demand for sweets led to its use by cake shops in the making of pastries. Prior to the 19th century, all candy work was done by hand with a resulting nonuniformity of product and smallness of production. The great Exposition of 1851 in London led to the modern development of the industry. The new candy-working machines put on display by the English manufacturers showed the rest of the world what was being done in that field and paved the way for the growth of the candy industry in Germany, France, and Holland. In the United States the making of candy is an important industry. In 1941 this industry sold over 2,500,000,000 lbs. of products with an estimated value of $403,000,000. Table 193 shows the trend of confectionery sales for the past 14 years. For comparison purposes this trend is shown in Fig. 73 as a graph compared to a base year (1929) taken as 100%.

TABLE 193

ESTIMATED TOTAL PRODUCTION OF CANDY IN THE UNITED STATES, AND MANUFACTURER'S AVERAGE VALUE PER POUND[a]

(Includes Confectionery and Competitive Chocolate Products)

Year	Millions of pounds	Per capita consumption, lbs.	Mfg. sales value, millions of dollars	Average value, cents/lb.
1927	1733	14.7	401	23.2
1928	1756	14.7	403	23.0
1929	1902	15.7	413	21.7
1930	1789	14.5	368	20.5
1931	1621	13.1	299	18.5
1932	1625	13.0	238	14.6
1933	1631	13.0	226	13.9
1934	1816	14.3	260	14.3
1935	1898	14.9	281	14.8
1936	2049	16.0	308	15.1
1937	2010	15.5	321	16.0
1938	1955	15.0	298	15.2
1939	2050	15.7	308	15.0
1940[b]	2225	16.9	336	15.1
1941[c]	2536	19.2	403	15.9

[a] U. S. Dept. Foreign and Domestic Commerce, Econ. Series, *Bull.* **13** (1940).
[b] Preliminary.
[c] Source: Confectionery Sales and Distribution (1941).

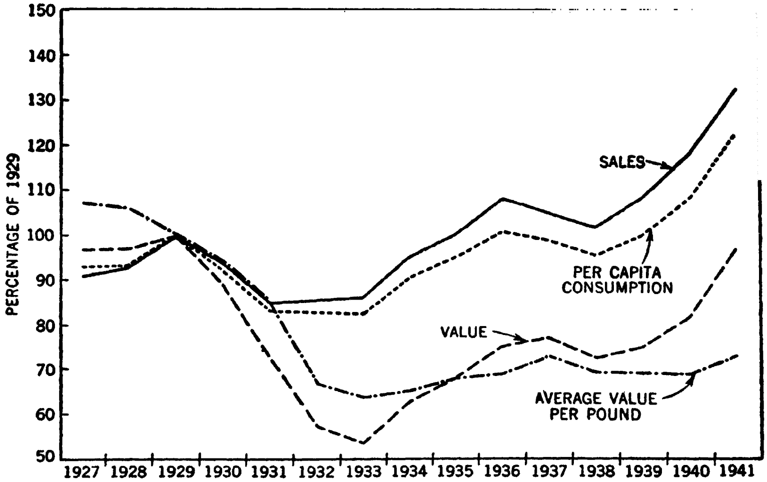


Fig. 73.—Estimated annual sales, annual production, per capita consumption, and average value of candy in the United States.

1. Candy or Confectionery

In the United States the two terms are synonymous. According to the Pure Food and Drug Act, "Candy is a product made from a saccharine substance or substances with or without the addition of harmless coloring flavoring or filling material, and contains no terra alba, barytes, talc, chrome yellow, or other ingredients, deleterious or detrimental to health, or any vinous, malt, or spirituous liquor or compound, or narcotic drug." A great diversity of materials is employed in the manufacture of

TABLE 194

PROXIMATE COMPOSITION OF FOOD INGREDIENTS USED IN CONFECTIONERY[a]

Ingredient	Fuel value, cal./100 g.	Protein, %	Fat, %	Carbohydrates, %	Ash, %
Almonds	640	18.6	54.1	19.6	3.0
Coconuts (dry)	579	3.6	39.1	53.2	0.8
Chocolate (bitter)	570	5.5	52.9	18.0	3.2
Chocolate (sweet)	516	2.0	29.8	60.0	1.4
Chocolate (sweet milk)	542	6.0	33.5	54.0	1.7
Cocoa (average)	329	9.0	18.8	31.0	5.2
Corn starch	365	9.1	3.7	73.9	1.3
Cream (heavy)	337	2.3	35.0	3.2	0.5
Dairy butter	733	0.6	81.0	0.4	2.5
Eggs (total edible)	158	12.8	11.5	0.7	1.0
Fruits (fresh)					
Apples (edible portion)	64	0.3	0.4	14.9	0.29
Lemons (edible portion)	44	0.9	0.6	8.7	0.54
Peaches (edible portion)	51	0.5	0.1	12.0	0.47
Pears (edible portion)	70	0.7	0.4	15.8	0.39
Oranges (edible portion)	50	0.9	0.2	11.2	0.47
Pineapples (edible portion)	58	0.4	0.2	13.7	0.42
Figs (dried)	300	4.0	1.2	68.4	2.4
Raisins (seedless and seeded)	298	2.3	0.5	71.2	2.0
Gelatin (plain, dry)	343	85.6	0.1	0.0	1.3
Milk					
Whole	69	3.5	3.9	4.9	0.7
Condensed	327	8.1	8.4	54.8	1.7
Evaporated	139	7.0	7.9	9.9	1.5
Skim	36	3.5	0.2	5.0	0.8
Milk (dried)					
Whole	496	25.8	26.7	38.0	6.0
Skim	359	35.6	1.0	52.0	7.9
Milk (malted)	418	14.6	8.5	70.7	3.6
Nuts					
Filberts	670	12.7	60.9	17.7	2.7
Peanuts (roasted, edible portion)	600	26.9	44.2	23.6	2.7
Pecan	747	9.4	73.0	13.0	1.6
Walnuts (edible portion)	702	15.0	64.4	15.6	1.7
Sugars					
Cane or beet	398	..	..	99.5	...
Corn (refined dextrose, anhydrous)	398	..	..	99.5	...
Maple	360	..	..	90.0	0.9
Brown	382	..	..	95.5	1.2
Sirups					
Cane	268	..	..	67.0	1.5
Corn (commercial)	322	..	..	80.6	...
Maple	256	..	..	64.0	0.7
Sorghum	268	..	..	67.0	2.5
Honey (strained or extracted)	319	0.3	0.0	79.5	0.2
Molasses (light)	260	..	..	65	3.0

[a] Adopted from U. S. Dept. Agr., *Circ.* 549 (1940)

candy; chief among them are sugar, cacao products, corn sirup, dairy products, peanuts, coconuts and other nutmeats, fruits, licorice, gum arabic, gelatin, cooking starch, molasses, essential oils, pectin, and mineral salt.

2. Cacao Products and Candy

Since enormous quantities of cacao products are used as a flavoring ingredient in the manufacture of candy, it is natural to link the original cacao products with candy.

Table 195

Raw Materials Consumed in the Confectionery Industry—1939[a]

Raw material	Lbs.	Value
Sugar, total	758,399,188	$34,521,774
Beet	256,399,250	11,607,814
Cane	451,882,122	20,898,558
Corn	22,028,334	828,592
Invert (Nulomoline), etc.	28,089,482	1,186,810
Corn sirup	502,275,200	12,996,268
Corn starch	22,973,895	742,874
Cacao beans and cacao products		
Chocolate coating	209,672,911	21,761,816
Chocolate liquor	13,444,540	1,280,068
Cacao butter	16,608,186	2,078,902
Cocoa powder		289,940
Cacao beans	5,690,048	335,208
Dairy products		
Milk (fluid, condensed, evaporated, and powdered)		3,544,498
Cream (butter fat)	2,695,283	460,310
Creamery butter	2,067,980	584,542
Coconut (shred, thread, etc.)	30,279,180	2,314,379
Gelatin	2,201,672	897,379
Nuts and peanuts	252,143,791	24,202,496
Chicle	233,402	78,906
Crude gums (used in chewing gums)	698,784	107,434
Essential oils	....	727,108
Flavoring extracts	....	1,340,411

[a] U. S. Dept. Commerce, Bur. of Foreign and Domestic Commerce, Econ. Series, *Bull.* **13**, Appendix F (1940).

Figures reported by 1108 establishments whose value of products amounted to $282,523,815, or 94.9% of the total value of products for the industry.

Note: Table 195 showing the materials consumed in the confectionery industry as reported to the Bureau of Census does not include substantial amounts of raw materials used in the production of 209,000,000 lbs. of competitive chocolate products by the chocolate and cocoa products industry in 1939. The cost of raw materials used in the production of competitive chocolate products in the chocolate industry amounts roughly to $25,000,000 and accounts for large quantities of sugar, cacao beans, milk products, and edible nuts. Much of these raw materials shown are used in the manufacture of chocolate coating and cacao butter produced by the chocolate and cocoa products industries for sale to confectionery industries.

However, there is a distinct difference between the two. Candy may be considered as a processed food made by mixing together various other food products. Cocoa and chocolate are natural foods having certain inherent food values and properties.

II. INGREDIENTS IN CANDY

1. Food Value

The fact that candy is considered a synthetic food does not in any way detract from its food value. Candy has a definite place in our diet. Besides meeting the human body's need of "sweets," it also furnishes significant fuel values. This is shown in Table 194, where the proximate composition and fuel value of the various candy ingredients are given.

TABLE 196

PRINCIPAL MATERIALS CONSUMED IN THE CHOCOLATE AND CACAO PRODUCTS INDUSTRY BY KIND, QUANTITY, AND COST—1939[a]

Material	Lbs.	Cost
Cacao beans	544,010,374	$28,547,394
Sugar		
Beet	33,428,641	1,446,509
Cane	223,595,338	9,794,129
Corn	1,662,383	68,697
Corn sirup	8,315,170	218,505
Creamery butter	1,372,451	345,782
Milk (fluid, etc.)	......	6,119,119
Nuts	......	2,989,722

[a] U. S. Dept. Commerce, Bur. of Foreign and Domestic Commerce, Econ. Series, *Bull.* **13**, Appendix F (1940).

The importance of the confectionery industry to our national economy is shown by the quantities of raw materials used by it in producing the different products. These values are given in Tables 195 and 196.

2. Sugars and Sirups

The confectionery industry is an important consumer of sugar and sweetening agents. In 1941 candy products accounted for 956,000,000 lbs. of sugar, while chocolate and cocoa products used another 338,000,000 lbs. Figure 74 shows the distribution of sugar by industries in the United States for 1941. Sugar (sucrose) is obtained both from sugar cane and sugar beet sources. The confectionery industry's consumption of sugar in 1941 was equivalent to about 60% of the total United States production of cane sugar and about 10% of the total United States beet-sugar production. Most of the sugar used by the industry is imported and was, of course, affected by the 1943 shipping shortage. For 1942 sugar was rationed at 70% of 1941 use.

Part of the sugar shortage has been relieved by the use of corn sweeteners, particularly corn sirup, and honey. There is a limit, however, to the quantity of corn sweeteners that may be used in place of sucrose. The amount of corn sirup used in candy making is dependent upon the quantity of sucrose used in the formula.

(*a*) *Corn Sirup*

The principal sweetening agent used in the making of confectionery is cane sugar or sucrose, a disaccharide. Other sugars play an important part in the manufacture of candy. Corn sirup, often called liquid glucose, or commercial glucose has become an indispensable ingredient. Its use is not confined to any particular branch of candy work. It may be used for the making of creams, so-called chewing candies, center confections, gums, and jellies. The value of corn sirup is due to the different components of the sirup.

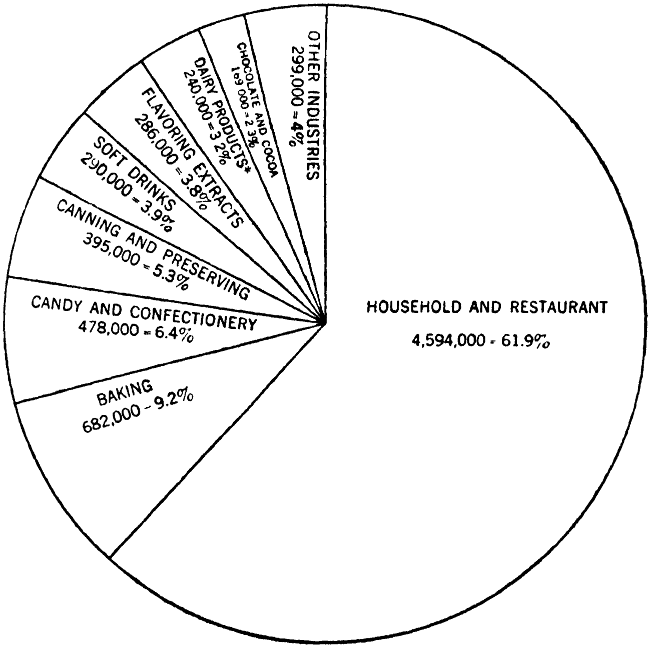


Fig. 74.—Estimated consumption of sugar in continental United States, 1941. (Short Tons, Raw Value) (Courtesy of U. S. Dept. of Commerce).
* Includes ice cream. Total 7,433,000 short tons.

The sirup is the result of the inversion, by an acid such as hydrochloric acid, of starch. Maize starch is used in the United States, potato starch in Europe. The discovery of the making of corn sirup is credited, indirectly, to the Napoleonic Wars. The English blockade of the European continent resulted in a shortage of many commodities, including sugar. A German chemist, Kirchhoff, tried to produce a substitute for gum arabic by hydrolyzing starch. In addition to his dextrin he also obtained a sweet-tasting substance that the French chemists were later able to offer as a sugar substitute. It is a melancholy fact that the stress of war is often productive of newer and better uses of materials.

In the manufacture of the sirup, a suspension of the starch is treated with hydrochloric acid (or sulfuric acid) with heat and pressure. The reaction is stopped before total inversion is obtained. The solution is then decolorized and evaporated to the proper consistency. Analyses of the finished product show that the main ingredients present are glucose, maltose, and dextrin. Evidently the name, liquid glucose or glucose sirup, is erroneous. See page 64.

The presence of dextrin makes the sirup an inhibitor to the graining of sugar products during cooling and for that reason has come to take the place of other chemical "doctors," cream of tartar or acid, which are used for that control. Because of the low solubility of glucose, compared to the solubility of sucrose, its presence is valuable in fondants and marshmallows. The soft, very smooth dextrose particles formed in these confections, instead of the hard cane-sugar crystals, permit a greater holding of the dissolved sugar mixture. Owing to the lower solubility of dextrose (glucose), the sirup phase in contact with it can take up more of the other sugars. Fondants made with corn sirup have been found to contain 84% sugar in their liquid phase as against 67% sugar in the liquid phase of fondants made only with cane sugar.[1] This increase of sugar content aids in preventing the fermentation of the fondant as well as its drying out.

Since corn sirup is a variable mixture it is difficult to predict its action merely by determining its chemical composition. The best procedure is to determine the effect of a particular batch of sirup under regular plant conditions. The sirup should not produce more than $8^1/_2$% inversion of cane sugar during a small batch run. A great deal of commercial corn sirup that is sold is not water-white but is colored by the addition of other sirups and materials, such as refiners' sirup and molasses.

(*b*) *Dextrose* (*Glucose*)

There is on the market a solid form of dextrose, known as Cerelose. Cerelose is considered, chemically, to be α-D^+-glucose. This product is also obtained from corn starch, but in its production the hydrolysis of the starch is allowed to go to the complete production of dextrose (about 94%). The dextrose is crystallized from the mother liquid under pressure and separated by centrifugal machines. Hydrated crystals are obtained from solutions at 38° C., anhydrous crystals from solutions at 45° C.

(*c*) *Invert Sugar*

Another inversion product that is used as a "doctor" is invert sugar, commercially called Nulomoline. This is obtained by the inversion of a 96% sugar solution. The action takes place in solutions adjusted to a *p*H of 3-4 by HCl through the use of invertase. The acid is then neutralized, with sodium carbonate, to a *p*H of approximately 6. The dextrose crystallizes and the entire mass is beaten when it reaches this crystalline equilibrium. This beating action results in a creamy product. It is sold in a semiplastic form.

---

[1] H. R. Jensen, *Chemistry, Flavouring and Manufacturing of Chocolate Confectionery and Cocoa.* Blakiston, Philadelphia, and J. and A. Churchill, London, 1931, p. 229.

(*d*) *Sirups Obtained from Sugar Cane*

Cane Sirup.—Cane sirup, or top sirup, is obtained by simply boiling the whole juice of sugar cane to a sirup consistency. It has a flavor similar to that of molasses, but in a high-quality sirup there is a delicacy and fineness of flavor that molasses lacks. Cane sirup must not contain more than 30% of water and not more than 2.5% of ash or mineral matter. The manufacture of sirups is discussed in Vol. II, Chapter XVII.

Golden Sirup.—Golden sirup is made during the refining of raw cane sugar. It is made by mixing the mother liquor, obtained after the second or third massecuite stage in the refining of sugar, with invert sugar.

Molasses.—This, in addition to the golden sirup, is a by-product of the sugar industry. Because of modern methods for refining sugar, the quantity of molasses produced, compared to the cane-sugar yield, has decreased with a corresponding decrease in sugar content and increase in nonsugar content. Molasses, because of the presence of gums, minerals, and nitrogenous matter, is not as "one-sided" a food as pure cane sugar. The standards for molasses are such that the total solids must be at least 75% and its ash not more than 5%. It contains also some of the lime and sulfur dioxide and possibly other materials used in the manufacture of sucrose. These leave their mark on the molasses flavor.

There are many types of molasses having their own peculiar flavor and name. Two of the better known are Barbados and blackstrap molasses. The former is considered a high-grade molasses, having a distinctive rumlike flavor. The latter is the lowest grade of molasses from which all the possible crystallizable sugar has been removed. It is used mainly in stock feed and for the production of alcohol.

Refiners' Sirup.—Refiners' sirup is the residual liquid product obtained in the process of refining raw cane sugars. It contains not more than 25% of water and not more than 8% of ash. It varies from dark brown to almost colorless, with a cane-sirup taste which is, however, not as strong as that of molasses.

Sugar Sirup.—Sugar sirup is the product made by boiling down sugar and water to a sirupy consistency. It must not contain more than 35% water. It cannot be labeled cane sirup, even if made from sucrose obtained from the juice of the sugar cane, because it is impossible to distinguish between beet sugar and cane sugar.

(*e*) *Sorgo*

Sorgo is a sirup made from the juice of the sorghum plant, which is related to the sugar cane. This sirup resembles cane sirup in flavor but has a sharper tang.

(*f*) *Maple Sirup*

Maple sirup is the product that is prepared by the evaporation of maple sap or by the formation of a solution of maple sugar. In order to conform with federal specifications, it must not contain more than 35% of water and the finished product must weigh at least 11 lbs. to the gallon.

Maple sap obtained from the sugar maple tree is poured into slightly tilted flat pans so corrugated that the flow of the sap is retarded. Fires under the pans cause evaporation and produce the maple sirup which is mainly sucrose but is characteristically flavored by substances contained in the sap. If the sirup is further concentrated until it crystallizes as brown sugar, maple sugar is obtained. A good tree will yield about 2 lbs of maple sugar or a quart of sirup per year.

(g) *Honey*

The use of honey as a sweetening agent goes back to ancient times Its principal value in candy is as a flavoring material in centers, toffees, and special creams. Commercial honey is the product of a special type of bee, called the honeybee (*Apis mellifica* and *A. dorsata*). The nectar of flowers, containing 20% sucrose, is converted by the enzyme of the bee into honey. Honeys derived from clover and alfalfa usually have the finest flavor. Because of the large-scale production of cane sugar and corn sirup with a resulting low price, the relatively high price of honey has made it a luxury. As a result, honey may be found adulterated with the cheaper sugars. The presence of added cane sugar or corn sirup or both may be detected by the usual analytical methods. Genuine honey contains sufficient fructose (levulose) to give a levorotatory reading in a polariscope determination, while cane sugar and commercial glucose are dextrorotatory. Adulteration with invert sugar, however, is difficult of detection since the composition of invert sugar is similar to the composition of the sugars in honey.

TABLE 197

COMPOSITION OF CONFECTIONERY SWEETENING AGENTS

Sugar product	Total sugar, %	Sucrose, %	Dextrose, %	Levulose, %	Invert sugar, %	Maltose, %	Dextrin, %	Ash, %	Water, %	Sweetening power, sucrose = 100[a]
Sucrose	100	100								100
Corn sirup	47 63		21 19			26 44	34 68		17 65	30[b]
Corn sugar (Cerelose)	91		87 5			3 5	0 5	0 04	9 1	66
Invert sugar (Nulomoline)	80	6			74				20	98
Golden sirup	68 5	31			37 5			3 9	22 5	77
Molasses	70 36	53 6	8 76	8				4 0	20	74
Honey	76 8	1 9	34 48	40 50				0 18	17 7	97
Maple sirup	64 07	62 6			1 47				35	64
Sorghum sirup	63	36			27			2 5	23	69
Saccharin										30,000–50,000
Sucrol										20,000

---

[a] Based on the composition of the sugar products and the relative sweetness values for the different sugars as determined by Biester *et al*

[b] The sweetening power of corn sirup varies according to the grade of the sirup, *i e*, degree of hydrolysis : high purity sirup = 40–45; low purity sirup = 26–29; "Sweetose" = 56–64.

(*h*) *Composition of sugars and sirups used in the making of candy*

The composition of the various sugars and sirups discussed above are summarized in Table 197. The relative sweetening powers of the different substances are also included. These sweetening powers were calculated from the composition of the sugar or sirup and based on the relative sweetness or sugars as given by Biester *et al.*[2]

3. Pectin and Other Gums

Scientific control in the making of confections and foods has led to an increased use of the various vegetable gums. They are invaluable as binders, fillers, stabilizers, and coatings. Gums are usually amorphous, transparent, or translucent substances of wide distribution among plants; they form sticky masses with water and are insoluble in organic solvents.[3] Gums have the advantage of being, in general, odorless and tasteless. The gums may be divided into two classes: those that yield a clear solution with water and those that swell in water and are only partially soluble. Those of the first class are considered real gums and those of the second are called vegetable mucilages.

Some of the more common gums are:[3]

Plant and Tree Exudates: acacia (arabic);* ghatti (British Indian Gum);* karaya (Sterculia Indian gum);† tragacanth (bassorin).†
Seaweed Extracts: agar-agar;† Irish moss (*Chondrus crispus*).*
Seed Extracts: locust kernel;† locust bean; quince seed.*
Fruit and Vegetable Extracts: pectin.†

The single asterisk indicates that the product is a true gum and is water-soluble. The dagger identifies the product as being partially soluble and as swelling in water.

Gums and mucilages occurring in plants are high polymeric polysaccharides, in which hexose, pentose, and uronic acid units are linked with one another.[4] Very little is known of the mode of linkage of these units and as a matter of fact the knowledge about the units themselves is incomplete.

(*a*) *Pectin*

Although most of the modern studies of the constitution of pectin have been made since 1917, its discovery dates back to the 19th century. Pectin is rapidly replacing starch, agar-agar, gum tragacanth, and gelatin, in the United States, for making jellies, Turkish pastes, and similar confections, besides finding many other important uses.

The term "pectin" is applied to a group of substances occurring in plants which have the common property of causing gelatinization, or jellying, of fruit juices, or water extracts of fruit pulp in which they are present or to which they may be added. Chemically the pectins are closely related to a mother substance known as pectose (also called protopectin). Pectose, a highly complex and insoluble compound, is present in practically all parts of green plants and is, relatively, most abundant in young rapidly growing parts, in fleshy roots such as those of the carrot, beet, turnip, and artichoke, also in such fruits as apples, quince, pears, currant, and gooseberry. In combination with the pectose is also found insoluble calcium pectate. As the fruit becomes mature, these compounds absorb water, swell, and pass into a gelatinous condition in which they are slightly soluble in water.

---

[2] A. Biester, M. W. Wood, and C. S. Wahlin, *Am. J. Physiol.*, **73**, 387 (1925).
[3] M. B. Jacobs, *Chemical Analysis of Foods and Food Products.* Van Nostrand, New York, 1938.
[4] K. H. Meyer, *Natural and Synthetic High Polymers.* Interscience, New York, 1942.

The term pectin is applied to the gelatinous, water-swollen substance or mixture resulting from this change.[5]

Pectin is considered to be, essentially, a long-chain, partially methylated, polygalacturonic acid molecule having a very large molecular weight. A high-grade natural pectin may have a molecular weight of over 200,000. This means that over 1000 units may be strung together to form each molecule.[6]

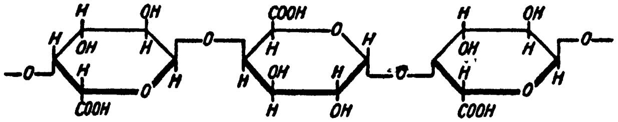
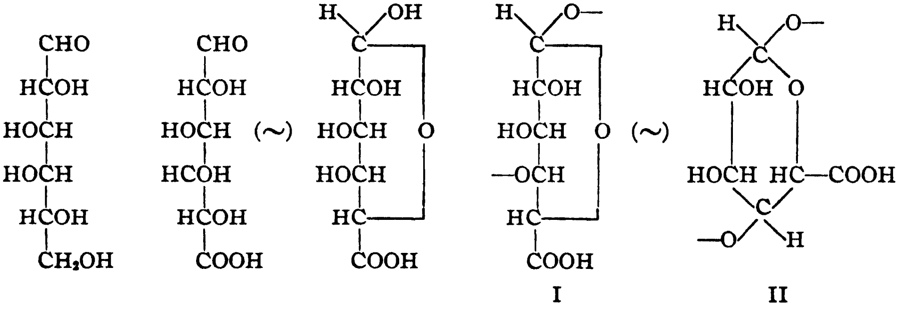


Fig. 75.—Formula of polygalacturonic acid from pectin showing three units of the molecule.[7]



(a) Galactose (b) Galacturonic acid (c) Anhydrogalacturonic acid

Fig. 76.—Relationship of galactose to anhydrogalacturonic acid.[8]

According to Figs. 75 and 76, the units of the long chain are 6-member rings composed of 5 carbon atoms and 1 oxygen atom, that is, furane rings. The characteristic side chains are methyl esters, $-COOCH_3$, or the corresponding acid, $-COOH$.

---

[5] J. S. Caldwell, Washington Agr. Expt. Sta., *Bull.* 147 (1917).

[6] C. W. Kaufman, E. R. Fehlberg, and A. G. Olsen, *Food Industries*, 14 (12), 57 (1942).

[7] K. H. Meyer, *Natural and Synthetic High Polymers*. Interscience, New York, 1942, p. 367.

Completely demethylated molecules of pectin, containing nothing but free acid groups, would be designated as pectic acid. Pectic acid has a very poor jellying power. Products containing both ester and acid groups are known as pectinic acid. Analysis indicates that commercial pectins are partially methylated.[5]

Commercial pectin is marketed either in the form of a concentrated solution or as a dry powder. Solutions are prepared by evaporating and clarifying fruit extracts. The addition of dilute acids will accelerate the extraction process. Powdered products are prepared by evaporating pectin solutions to dryness or by precipitating pectin from fruit extracts. The commercial sources of pectin are the citrus fruits and apple waste materials. Pectin is a white amorphous solid; its specific gravity is 1.18 at 20° C.; it decomposes at 158° C. after turning brown at 156° C.

TABLE 198

PERCENTAGE OF PECTIN IN CERTAIN MATERIALS[a]

Material	Pectin in fresh material, %	Pectin on water-free basis, %
Apple pomace	1.5–2.5	15–18
Lemon pulp	2.5–4.0	30–35
Orange pulp	3.5–5.5	30–40
Beet pulp	1.0	25–30
Carrots	0.62	7.14

[a] C. P. Wilson, *Ind. Eng. Chem.*, **17**, 1066 (1925).

A list of the different uses of this gum follows:[8]

1. In the making of jams, jellies, marmalades, candy, and similar products.
2. For glues and mucilages.
3. For sizing textiles.
4. In the making of lacquers and explosives similar to the nitrocellulose and nitroacetate type.
5. For the stabilization of oil-water emulsions; of particular value for such preparations as cod-liver oil, mayonnaise, and essential oils.
6. For the stabilization of salves, nasal jellies, hand lotions and hair preparations.
7. To increase the quality of process cheese by adding to its water-holding capacity.
8. To improve the texture and increase the yield of bakery goods.
9. To make dehydrated fruit juices and beverages.

(b) *Gum Arabic*

Because of its thickening power and its ability to prevent crystallization, gum arabic is of particular value in the confectinery industry. The gum is collected, as an exudate, from the stems of the acacia tree (*Acacia verek* or *A. senegal*) which grows wild in desert regions of the Anglo-Egyptian Sudan or French Sudan as well as in other parts of Africa and Arabia.[9]

---

[8] *Manufacture, Properties and Uses of Pectin.* Compiled by William E. Elwell; directed by W. H. Dehn; published by Belle Reeves, Secy. of State, Washington, W.P.A. Project No. 2839 (1939).

[9] J. A. Ridgeway, *Can. Chem. J.*, **4**, 330 (1920).

Good samples of gum arabic dissolve almost completely in cold water leaving an undissolved portion of from 1 to 2%.

Investigation of the nature of the gum indicates that it is not a substance of a definite formula. Its general composition, however, can be indicated by a nucleus acid, probably galacturonic acid, to which is linked arabinose.[10]

(c) *Gum Tragacanth*

Gum tragacanth has important uses as a thickener in food products and as an emulsifying agent in certain pharmaceutical preparations. Because of its high cost, however, other gums, including locust kernel, have been substituted for it.

Tragacanth is an incision product from Asiatic shrubs of the genus *Astragalus*.[11] Although information about its structure is meager, it has been found to contain a considerable amount of uronic acid (50%) in addition to a considerable amount of arabinose (about 49%).[12]

(d) *Agar-Agar*

Agar is the commercial name applied to the dried and more or less purified stems of certain kinds of marine plants of the species *Gelidium algae.* Most of the agar of commerce comes from China, Japan, Malaysia, Ceylon, and neighboring coasts. In China it goes by the name of "Hai-thio" or "Ta-O," in France it is known as "gelose"; it is synonymous with Bengal or vegetable isinglass.[13]

Agar is often confused with carragheen or Irish moss. This gum is derived, mainly, from *Chondrus crispus*, an alga which is very abundant in the North Sea. It produces a gel which is very similar to agar.

There are two methods of preparation. One method consists merely in drying and bleaching the thallus of the algae, in the sun. Such a product contains many impurities such as diatomaceous refuse and other mineral and vegetable matter foreign to the plant. Another method consists in making a jelly of the seaweeds, allowing the water to freeze out, and finally cutting the residue into thin strips and drying thoroughly.[13]

Investigators describe the agars as polymannuronic acids[14] with the possibility that their molecular weights may be similar to that of cellulose.[15]

The use of agar is not confined to any one field. It is used in the making of soups, jellies, pharmaceutical emulsions, sherbet and water ices, ice cream, meat and fish pastes, in the canning of fish for transportation when a fragile fish is packed in jelly, in the clarification of liquids, as a stabilizer in malted milk drinks; and in bread to inhibit staling. Its incorporation in breakfast and health foods, as well as in pharmaceutical preparations, depends on its ability to act mechanically as roughage which stimulates peristalsis. In addition it is used in bacteriological technique and as a nonprotein colloid in colloid research.[16]

---

[10] Arthur G. Norman, *Biochem. J.*, **23**, 524 (1929).

[11] William Clayton, *Colloid Aspects of Food Chemistry and Technology.* Blakiston, Philadelphia, 1932.

[12] K. H. Meyer, *Natural and Synthetic High Polymers.* Interscience, New York, 1942, p. 376.

[13] C. R. Fellers, *Ind. Eng. Chem.*, **8**, 1128 (1916).

[14] W. L. Nelson and L. H. Cretcher, *J. Am. Chem. Soc.*, **54**, 3409 (1931).

[15] K. H. Meyer, *Natural and Synthetic High Polymers.* Interscience, New York, p. 376.

(*e*) *Locust-Kernel Gum*

The locust-kernel gum is slowly gaining in importance as more information of its properties is learned. At present it finds application in many fields. It is used by the tanning industry as a restraining agent; by the textile industry to size and finish yarns; as a color thickener in calico printing; and as a thickener of food sauces.

The gum is obtained from the locust or carob bean (*Ceratonia siliqua*). The seeds are separated from their shells by treatment in rollers and crushers. The endosperms are then heated in a rotary oven until they are golden brown in color. The roasted material is then allowed to swell by treatment with boiling water. The resulting viscous material is filtered; the clear filtrate is dried and finely ground. The substance is to be obtained commercially as a mealy white powder under the names of "Locust-kernel Gum" and "Gum Tragon," and also as a tough jelly, containing about 4% of solids under the name of "Tragasol."

Analysis of the gum indicates that its ultimate composition (C, 44.17; H, 6.32; O, 49.11; and N, 0.40%) agrees with the formula $C_6H_{10}O_5$, and that although the true gums, such as gum arabic and gum tragacanth, are acids of high molecular weight—a combination of a nucleus acid with various hexoses, pentoses, etc.—the gum obtained from the locust bean is a carbohydrate composed of the anhydrides of the hexoses, mannose, and galactose, and probably the hemicelluloses.[17]

Locust-kernel gum swells in cold water and, on stirring, separates into lumps which do not break down readily. It must be allowed to stand for a considerable length of time before sufficient water is absorbed to form a homogeneous mixture.

4. Gelatin

Gelatin has many properties that are common to the plant gums, although it is a protein. It is a natural high polymer, having molecular weights (for commercial gelatin) from 20,000 to 70,000; solutions of gelatin will set under certain conditions to form translucent gels.

Gelatin has wide uses in the confectionery industry; in the making of table jellies, pastilles, gums, ice cream, and marshmallows. The function of this compound varies because of its different properties. It may be used because of its elastic consistency; because it has the property of holding air and water; or because it can inhibit the crystallization, or graining, of sugar.

---

[16] William Clayton, *Colloid Aspects of Food Chemistry and Technology*. Blakiston, Philadelphia, 1932.

[17] A. L. Williams, *Analyst*, **53**, 411 (1928).

Gelatin and glue are prepared from collagen-containing material (principally hide waste, tendons, and bones) by hydrolytic breakdown by boiling with water or by treatment with superheated steam. In the case of gelatin a product is required which shall be pure and odorless and which will gelate in aqueous solution. In the case of glue, value is laid on the adhesive properties.

The composition of gelatin, as given by Sweetman,[18] is as follows: protein, 85–86%; ash, 1–2%; water, 13–14%.

The composition of gelatin obtained by hydrolysis of collagen fibers is as follows (in per cent by weight):[19]

Glycine	25.5	Methionine	1.0
Alanine	8.7	Arginine	8.2
Leucine	7.1	Lysine	5.9
Serine	0.4	Histidine	0.9
Phenylalanine	1.4	Proline	19.4
Glutamic acid	5.8	Oxyproline	14.1
Aspartic acid	3.3	Ammonia	0.4

Little is known of the arrangement of the amino acid units. See pages 163 and 164.

Gelatin does not have a definite melting point but softens at about 140° C. It is insoluble in cold water but absorbs from 5 to 9 times its own weight when ground and digested for 48 hrs. at 15° C. Solutions should not be heated above 60–70° C., or a progressive destruction of the gelation and the jellying power of the gelatin will result.

Because gelatin lacks the important essential amino acids, tryptophane and tyrosine, it cannot be considered as a source of protein for life-sustaining purposes. The chief food value of gelatin is its contribution to the palatability of the food mixtures in which it is employed.

5. Cacao

(a) *History*

When Cortez and his soldiers landed in Mexico in 1519, they found that the natives of that unfortunate land had a cultured and industrious civilization. The Aztecs had their own religion and mythology. Since Cortez was interested only in plunder, it was not long before this ancient civilization was destroyed and the country invaded by the conquering Spaniards. The search for valuable products, to be shipped back to Spain, directed their attention to the product of a certain tree that had been cultivated by the Indians for at least 1000 years. According to their mythology the seed of this tree had been carried from the New World version of the Garden of Eden into Mexico by Quetzalcoatl, God of Air. The fruit, it is related, was a favorite food of the gods. The great Swedish botanist, Linnaeus, later christened the fruit *Theobroma cacao,* Theobroma meaning in Greek "food of the gods." The Aztecs made a drink called "chocolatl" from the seeds of the fruit of this tree.

---

[18] M. Sweetman, *Food Preparation.* Wiley, New York, 1937.

[19] K. H. Meyer, *Natural and Synthetic High Polymers.* Interscience, New York, 1942, p. 455.

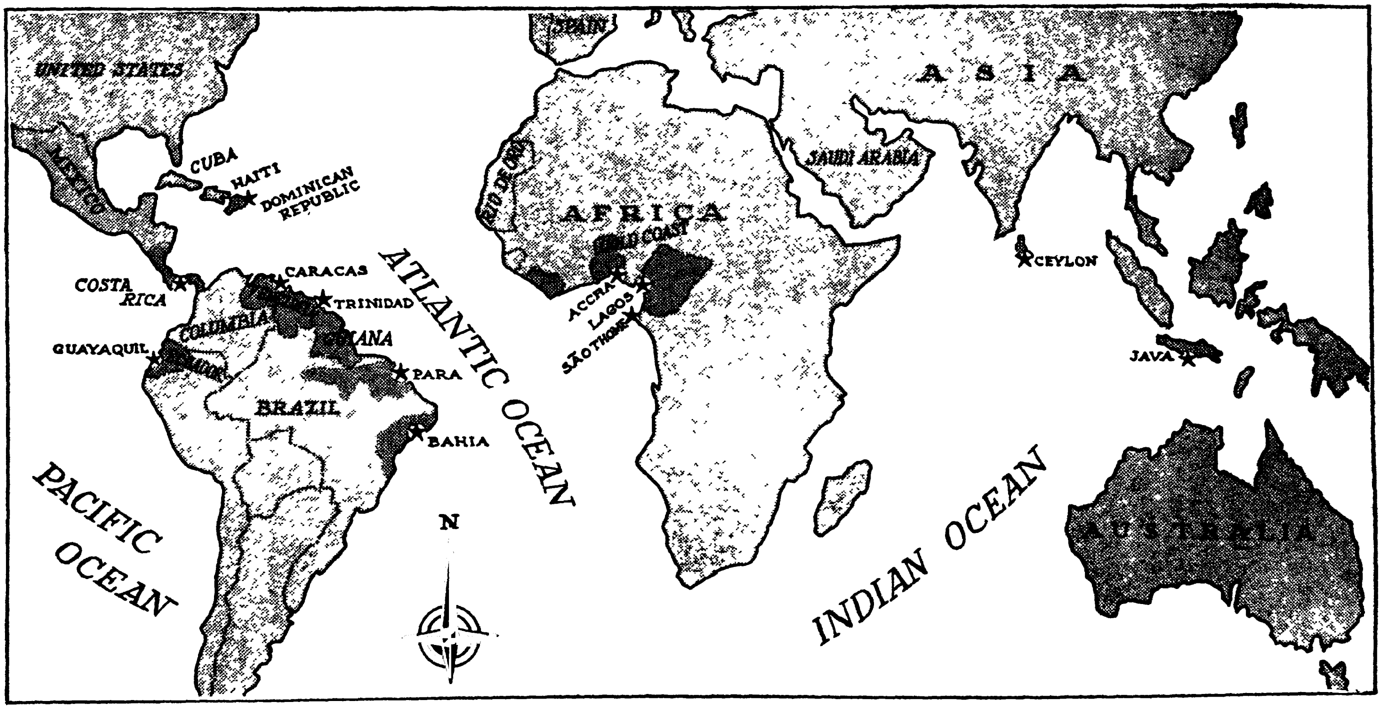


Fig. 77.—Sources of cacao beans. Stars indicate principal shipping ports. Dark shaded areas are cacao producing regions.

These seeds were considered very valuable and were used as a means of barter in the Aztec and Mayan civilization. A man's wealth was often judged by the number of cacao seeds (incorrectly called beans) he possessed. In Mexico, at that time, a good slave could be purchased for 100 beans.

It is interesting to note that cacao was introduced into Europe long before coffee and tea. Spain recognized its value and kept a monopoly on its use and production for almost a century. Then the use of the beverage rapidly spread throughout the rest of Europe. It was first introduced into Italy in 1606, and shortly afterwards into near-by Austria.

TABLE 199

EXPORTS OF CACAO FROM THE PRINCIPAL PRODUCING COUNTRIES[a]
(In Thousands of Long Tons[b])

Country	1932	1933	1934	1935	1936	1937	1938[c]	1939[c]	1940[c]	1941[c]
Gold Coast	234	236	230	269	311	236	257	275	...	...
Brazil	96	97	100	110	120	103	130	128	115	122
Nigeria[d]	71	61	78	88	81	103	96	114	...	...
Trinidad	19	23	12	20	13	12	19	7	7	8
Ecuador	15	10	19	20	19	21	18	13	11	13
San Domingo	17	20	23	28	18	19	28	28	23	19
Ivory Coast	25	30	41	43	49	47	53	50[f]	...	..
Venezuela	16	17	14	15	16	16	13	10	11	11
Cameroons[e]	14	17	19	23	24	26	26	25	25	...
Togo	6	7	6	11	10	10	8	9	...	...
Ceylon	4	3	4	3	3	4	4	4	4	...
Granada	4	5	4	4	4	3	4	4	3	4
Others	39	44	42	44	44	45	45	45[f]	...	...
Total	560	570	592	678	712	645	701	712[f]	...	...

[a] Imperial Economic Committee and official sources. *1942 Commodity Year Book.*
[b] One long ton equals 2240 lbs.
[c] Preliminary.
[d] Including Cameroons under British mandate.
[e] Under French mandate.
[f] Estimated.

The marriage of Anne of Austria to Louis XIII gave France its contact with the cacao beverage. All this time Spain maintained its grip on the production of the cacao bean. The price remained very high. In 1693 it is said that a certain Rudolphe de Canvillet possessed more cacao beans than any other individual in France. He had 10 lbs.! The total amount in the whole country at that time was 22 lbs. Cacao was introduced into England, Holland, and Germany during the middle of the 17th century.

The high food value, low cost of production, and many uses of cacao have resulted in its widespread growth in most countries situated in the tropical belt near the equator. A map showing the principal cacao-producing countries is given in Fig. 77. The world trade in cacao is of comparatively recent growth. In 1895 the total world exports were only about 77,000 long tons, with the main producing countries Ecuador, West Indies (Trinidad, Grenada, and San Domingo), Brazil, and Venezuela exporting about 73% of the total. The world exports almost doubled the next decade, and doubled again by 1915. In 1925 about 500,000 metric tons were exported. In 1939, the last year in which export statistics were available, the total world exports of cacao were estimated at 712,000 long tons, or, if you like large figures, 1,594,880,000 lbs. Table 199 shows cacao exports from the leading countries since 1927.

This remarkable advance in production was made possible by the introduction of cacao to West Africa. In 1905 British and French West Africa contributed only 4% of the total world exports in that commodity. In 1925 the Gold Coast alone furnished 44% of the world total. It can be seen that within a period of 20 years the center of production of cacao had shifted from South America and the West Indies to West Africa. What were once trackless African jungles, inhabited only by savage bushmen and wild animals, today are cacao plantations, operated for the most part by natives.

TABLE 200

APPARENT CONSUMPTION OF CACAO IN SELECTED COUNTRIES[a]

(In Thousands of Long Tons)

Country	1932	1933	1934	1935	1936	1937	1938[b]	1939[b]	1940[b]	1941[b]
United States	181.2	193.0	196.2	271.5	288.8	228.6	211.6	277.0	263.8	293.0
Germany	76.3	75.9	102.3	78.0	77.6	75.5	71.8	65.0	...	...
United Kingdom	66.9	66.6	72.3	82.4	102.1	94.2	94.4	110.0	...	...
Netherlands	39.7	46.2	52.9	60.9	65.9	52.4	73.8	75.0	...	...
France	43.3	41.3	39.6	42.7	46.5	40.7	41.3	50.0	...	...
Canada	8.0	10.7	9.2	10.7	15.9	10.9	14.8	17.0	17.0	26.8
Czechoslovakia	10.5	8.4	10.2	13.2	11.9	10.0	9.8	...	...	...
Italy	6.7	8.3	8.5	11.7	7.9	7.7	7.9	8.0	...	...
Belgium	9.2	7.0	8.7	9.1	9.9	8.6	9.8	10.0	...	...
Switzerland	5.3	7.4	7.1	7.5	7.8	6.4	8.8	10.0	...	...
Australia	4.8	6.1	6.2	5.7	6.5	6.4	7.9	8.0	...	...

[a] Imperial Economic Committee and official sources. *1942 Commodity Year Book.*

[b] Preliminary.

The largest importer of cacao in the world today is the United States, accounting for about 45% of the world total. Until 1935 Germany ranked second, but since then it has given place to England. Table 200 gives the consumption of cacao in selected countries.

The outlook for cacao products in the United States for 1943 depended upon the shipping problem then facing the entire world. For 1942, cacao was rationed in the United States at 70% of 1941 use. This was later reduced to 60% for the last quarter of 1942.

(*b*) *Cacao, Cocoa, and Chocolate*

As might be expected, the terms used for cacao and cacao products have become corrupted into different forms because of the international trade in this product. Somewhere, during the translation of Indian names into Spanish and from Spanish into French, Portuguese, German, and English, cacao became cocoa and the drink made by grinding cacao nibs with water and spices became chocolate.

In trade circles today the following terms are used: cacao, cocoa, and chocolate.

Cacao is used when referring to cacao beans. It is also the descriptive adjective for the tree, the pod, the flower, the seed shell, and the butter; for example, cacao tree, cacao shell, cacao butter. Incidentally, the cacao bean is correctly called cacao seed.

Cocoa is the commercial name given to the manufactured product for making the beverage.

Chocolate designates the manufactured product when it appears in solidified forms such as baking chocolate, sweet chocolate, milk chocolate, etc.

The standards for the different cacao products are discussed in Vol. II, Chapter XVIII.

Cacao is to be distinguished from the coca plant, source of the powerful alkaloid cocaine. Cacao butter, or cocoa butter, expressed from the cacao seeds, is not to be confused with coconut oil. Coconut oil is expressed from the coconuts of the trees *Cocos nucifera* and *C. butyracea.* Many texts add to this confusion of terms by using the spelling cocoanut oil for coconut oil. The characteristics of these fats are not the same.

(*c*) *Cacao Tree*

It is generally believed that cacao trees originated in the region of the basins of the Orinoco and the Amazon. They were evidently cultivated for centuries before being discovered by the Spaniards. The tree, *Theobroma cacao,* is the important member of a score of small trees that are indigenous to tropical America and belongs to the order *Sterculiacae.* It has been described[20] as a handsome perennial evergreen, of moderate size, bearing large, smooth and glossy elliptical leaves, growing principally at the ends of branches, but sometimes springing directly from the main trunk. The fruit when matured is the center of a cluster of the pink, almost colorless flowers that grow on the main branches and the trunk. The little pink flowers and the resulting pod grow most freely on the older branches and trunks of the trees, often on those entirely bare of foliage, instead of among the fullest foliage as with other fruits. Thousands of flowers appear on the trunk and branches of a mature tree—but it has been calculated that only one perfect fruit develops for each 400 flowers! This fruit is from 2 to 4 in. in diameter and from 7 to 12 in. long. The color of the ripe pod varies and may be yellow, orange, dark red, or purple. It has a hard, thick, leathery rind marked with 10 very distinct longitudinal ribs. The interior of the fruit contains from 25 to 75 seeds in 5 rows, imbedded in a soft white or pinkish pulp. These are the cacao or cacao beans of commerce. The cacao pod is generally 75% pericarp with placenta and pulp, and 25% fresh seeds or beans.

Varieties of Seeds.—Cacao may be divided into two main types—Criollo and Forastero.[21] The Criollo beans are obtained from native stock that had been cultivated in South America for thousands of years. These beans are considered more desirable than the Forastero type because of their delicate aroma and taste. The Criollo cacao are large and plump, have a sweet or only very slightly bitter taste. After fermentation these beans produce kernels having a white to pale brown color, and a fine flavor. Extreme care is needed in the cultivation of the Criollo bean; they require a full supply of water and are susceptible to many plant diseases. The production of this type of bean, because of its high cost, is diminishing in favor of the Forastero cacao. Venezuela is the principal grower of Criollo beans.

---

[20] J. F. Caius, *New Review,* 15, 211 (1942).

[21] C. J. J. van Hall, *Cacao.* Macmillan, London, 1932, p. 77.

Forastero beans are derived from a wild-growing variety of *Theobroma cacao.* It was originally called Trinitario in East Venezuela in order to distinguish it from the finer cacaos of the west. It is a stronger grower, hardier and crops easier than Criollo. The principal Forastero nursery is Trinidad. The Forastero cacao provides the greater part of the world's supply of cacao. There are several subvarieties of this bean. The most important one is called Amelonado. The kernels of the Forastero beans are usually purple as compared to the white or pale brown color of the Criollo seeds.

Cultivation of the Tree.—The cultivation of the tree *Theobroma cacao* is limited to tropical countries because of the conditions under which it can flourish. There must be a mean temperature of 80° F., a rainfall of 50 in. and upward, shelter from prevailing winds, rich deep well-drained soil free from inundation with salt water, and moderate shade at all times. In some countries the shade is usually obtained by planting, side by side with the cacao tree, other quicker-growing trees. One common variety of these protecting trees is called "Madre de Cacao" (mother of cacao). Formerly these protecting trees had no commercial value. Recently the trees from which Para rubber is obtained, genus *Hevea,* came into use. These trees yield a product whose value compares favorably with the trees they are protecting.

Cacao trees begin to bear at their third or fourth year, reach their prime in from 12 to 15 years, and then will continue to produce for some 50 or more years. The tree is an all-year-round fruit producer, is seldom barren of both fruit and flowers during any of the 12 months. There is an interval of about 5 months for the growth and development of a newly fertilized flower into a fully ripe and matured cacao pod. There are usually two crops a year. In the Gold Coast and Nigeria the main crop season is from September or October to January or February, while the small "mid-crop" (which accounts in the Gold Coast for only 5 to 10% of the total annual production) comes about 4 to 5 months later. Brazilian crops normally reach the world markets slightly before the African crops, and its peak exports occur usually between February and May. For statistical purposes the crop year is taken as from October 1 to September 30.

(*d*) *Fermentation of Cacao*

"The hidden flavor of the hidden fruit of a hidden tree" is an apt phrase when applied to cacao. The true chocolate flavor of the cacao is really obtained only after the bean has been fermented, cured, and roasted. The fermentation of cacao was not originally devised to improve the flavor; its first objective was the removal of the adhering pulp. Once the pulp was removed the beans could easily be dried and made ready for market. The process is an old one that has evolved naturally by trial and error. A careful scientific study has been made of the fermentation of cacao; however, the old method is still used. The main accomplishment of the investigators in this field has been to disseminate knowledge of the best procedures to be followed.

The main purposes of the fermentation are (*1*) to remove the adhering pulp from the bean proper, (*2*) to destroy the vitality of the seeds, and (*3*) to give aroma, flavor, and color to the bean. Although the fermenting of cacao is very complex and presents an almost endless number of problems to the scientist, the native proceeds with the job in the most casual manner.

TABLE 201

PERCENTAGE COMPOSITION OF FRESH PULP[a]

Component	Usual range
Water	80–90
Albuminoids, etc.	0.5–0.7
Glucose	8–13
Sucrose	0.4–1.0
Starch	Trace
Nonvolatile acids (as tartaric)	0.2–0.4
Iron oxide	0.3
Salts	0.4–0.5
Alcohol	Nil
Volatile acids	Nil

[a] A. W. Knapp, *Cacao Fermentation*. Bale, London, 1937.

The pods (fruit), after being carefully removed from the trees, are first sorted according to size and degree of ripeness. The selected pods are then quickly split open and the contents put at once to ferment. This "sweating" process occurs when the cacao beans and adhering pulp are covered with banana or plantain leaves, after being piled in perforated wooden sweating boxes. Experience has shown that the best fermentations are conducted in boxes which do not greatly exceed 1 m. (39 in.) in height and which have a volume not exceedingly 9 cu. m.[22] The time of fermentation will depend upon the type of bean, and will last from 3 to 12 days. The thin-skinned Criollo beans will take much less time than the thicker-skinned Forastero type. During the fermentation the beans must be stirred and turned frequently and the heat generated by the fermentation must also be controlled. The process can be divided into two stages. At first, the ripe pulp, which contains considerable sugar (see Table 201), begins to ferment due to the presence of specific *Saccharomyces* or yeasts. Carbon dioxide and alcohol are formed, other organisms, including *Mycoderma aceti*, will cause the oxidation of the alcohol to acetic acid. Since the reaction is exothermic the temperature of the fermenting mass is raised. The "sweatings" from the pulp will drain from the perforated boxes and be discarded. Proper aeration is essential at this point to prevent the growth of objectionable anaerobic organisms and to promote the vigorous growth of the yeasts. Air is also essential for the oxidation of tannins in a later stage. Stirring allows circulation of the air, helps to maintain a uniform temperature, and distributes the various yeasts and organisms evenly through the mass.

---

[22] Leonard J. Schwartz, U. S. Dept. Commerce, Trade Promotion Ser. **138** (1932).

The second stage of the fermentation follows from the fermentation of the pulp. The heat evolved, 44 to 50° C., will kill the cacao seed which will lead to important changes in the bean. The diastase of the kernels (cotyledons) attacks the starch in the seed to form sugar and dextrin. The cells become disorganized and allow the cacao pigment, present in the nonstarch cells, to diffuse throughout the bean. Theobromine, originally present in the fresh kernel, will partly diffuse to the cacao shell. Oxidase enzymes will split certain complex tannins with a resulting loss in astringency and change in color. These tannins and the effect of fermentation on them are shown in Table 202.

Table 202

Effect of Fermentation on Cacao Tannins[a]

Tannin Substances Present in the Fresh Cotyledons			
Colorless			Purple
Catechin	Tannin	Catechin Tannin	Cacao-Purple[b]
↓	↓	↓	↓
Red-brown products	Tannin Dark-brown products	Caffeine, Red-brown products	Cacao-Purple and brown products
Tannic Substances Present in Cotyledons after Fermentation and Drying			

[a] A. W. Knapp, *Cacao Fermentation.* Bale, London, 1937.

[b] Knapp uses the term cacao-purple in place of cacao-red as being more descriptive of the actual color.

The fermentation is considered finished when the temperature of the mass falls to 35° C. Some planters prefer to ferment to a definite color and appearance of the bean.

(*e*) *Curing*

The fermented bean contains about 33% of water. This moisture content must be reduced below 8% before it can safely be stored without becoming moldy. The drying operation is considered a continuation of fermentation. As the drying proceeds the color of the shell darkens and further oxidation of the tannins occurs. The drying methods fall roughly into three classes: (*1*) sun drying, (*2*) sun drying plus artificial heat, and (*3*) drying by artificial means. Natural-dried cacaos are preferred by the manufacturers. One danger of sun drying is the ease with which the cacao might become contaminated with mold spores. In countries where the rainfall is high it is necessary to use artificial drying methods. The drying process usually takes from 3 to 8 days; slow-dried beans are considered preferable.

TABLE 203

CHANGES IN FORASTERO CACAO[a]

Observation	Entirely unfermented (dried direct)	Half fermented (and dried)	Fully fermented (and dried)
Shape of bean	Flat	Flat	Plumper
Shell:			
Texture and fit	Soft and close fitting	Hard and close fitting	Crisp and more or less free
Color of section	Almost white	Light	Pale brown
Color, inside surface	Clean, pale	Clean	Showing extensive dark brown deposits
Germ	White section	Pale brown	Dark brown
Kernel (cotyledons):			
Color of section	Slate	Bright heliotrope or claret	Brown, brown-purple or brown tinged with purple
Color of surface	Dull, dark gray	Darker, a few show "rust"	Glossy, purplish brown, many show "rust"
Consistence	Leathery or cheesy	Very hard, woody, and tough	Crisp
Appearance	Solid	Solid	Open grained
Taste	Bitter and astringent	Bitter and astringent	Less astringent
Aroma			
Unroasted	Faintly earthy	Slightly acid	Acid, with faint fragrance
Roasted	Resembles broad beans (*Vicia faba*)	Like unfermented	Characteristic chocolate odor

TABLE 204

SKINNED BEANS (FORASTERO) STRAIGHT FROM POD[a]

Analysis	Approximate composition, %
Cacao butter	37.0
Moisture	33.0
Protein	6.0
Cacao starch	4.0
Mineral salts	2.2
Crude fiber	1.8
Cacao tannin, at least	1.6
Cacao catechin, at least	0.5
Theobromine	1.2
Caffeine	0.3
Unestimated (other carbohydrates, mucilage, pectin, etc.)	12.4

[a] A. W. Knapp, *Cacao Fermentation.* Bale, London, 1937.

(*f*) *Characteristics of a Good Cacao*

The quality of cacao depends upon many factors, including the particular variety of the bean and the care taken in its cultivation, fermentation, curing, and storage. No matter how good the original quality of the bean, unless it has been properly treated it will produce an inferior product. In 1937, the British Government, alarmed by the falling off of quality and production of cacao in the Gold Coast and Nigeria, enacted a series of "Buying Agreements" with the leading cacao buyers in Africa. Through these agreements Great Britain gained control of the entire crop. It was able to stabilize prices and do away with certain existing bad practices in the cultivation and preparation of the cacao for the market. Provisions were made for long-range buying and cultivation of crops. Native farmers were advanced money for extending or improving their farms. A commission, sent by England to investigate the results of these agreements in 1938, recommended their continuance.[23]

Table 205

Approximate Analyses of Fermented and Unfermented West African Beans[a]

Analysis	Unfermented and dried	Fermented and dried
Average weight of beans, g.	1.072	1.020
Nib, in cotyledons, %	89.60	88.56
Shell, %	9.63	10.74
Germ, %	0.77	0.70
Water, in cotyledons, %	3.65	2.13
Fat, in cotyledons, %	53.05	54.68
Total ash, in cotyledons, %	2.63	2.74
Nitrogen, in cotyledons, %		
Total nitrogen	2.28	2.16
Theobromine	1.71	1.42
Caffeine	0.085	0.066
Total carbohydrates, in cotyledons, %	14.21	17.43
Tannins, in cotyledons, %		
Tannic acid	2.24	1.99
Cacao-purple and cacao-brown	5.30	4.16

[a] Adopted from A. W. Knapp, *Cacao Fermentation.* Bale, London, 1937.

According to Knapp,[24] the kind of cacao desired by manufacturers should have the following desirable characteristics:

(*1*) The beans should be large (approximately less than 400 to the pound), healthy, even in size, and plump. The shell should be unbroken, with the kernel loose in the shell.

(*2*) The shell should be crisp and tough, but not too friable, and more or less detached from the cotyledons. On pressing powerfully with the thumb, a bean, held in the palm of the hand, should break readily into a number of crisp nibs.

(*3*) The odor should be clean, pleasant, and characteristic. It is usually vinegary. There should be no trace of foreign odor, whether mold, musty, or smoky. The true chocolate odor is really only determinable after roasting.

(*4*) The fragments should taste fatty, refreshing, not too bitter or astringent, nutty, and neither harsh nor sour.

---

[23] Great Britain, "West African Cocoa," Commission on Marketing, H. M. Stationery Office, London, 1938.

[24] Great Britain, *Imperial Institute Bull.*, **31**, 360 (1933).

(*5*) If the bean is cut lengthwise through the center it should be fairly open-grained. The color of the section should be cinnamon-brown, dark brown, purple-brown, according to the variety, but should be free from mud or slate color.

(*6*) The following precautions should be taken when preparing the beans for the market: (*a*) Cacao should be gathered when in ripe condition; unripe beans are small and generally ferment imperfectly. Overripe beans tend to break and become moldy and grubby. (*b*) Beans should be properly fermented, although an unfermented bean may later be improved by fermentation; an underfermented bean will yield an inferior product. (*c*) It is not desirable to wash beans after fermentation because of the danger of cracking the shells. (*d*) The beans must be dried thoroughly to prevent mold.

TABLE 206

CHARACTERISTICS OF INDIVIDUAL CACAO VARIETIES[a]

Variety	Source	Description
F. F. Accra	Africa	Forastero type, amelonado variety, dark brown to purple color, has a mildly acid to harsh bitter taste. Used chiefly as a base for cheap chocolate and cocoa. Price New York market 4.5 cents per lb.[b]
Arriba	Ecuador	Forastero, fine strong flavor and unique scented aroma, rich dark brown color. Price 8.0 cents[b]
Bahia	Brazil	Forastero, smoky red-brown in color, harsh flavor, similar to Accra. Price 4.5 cents[b]
Puerto Cabello	Venezuela	Criollo, rich brown in color, sweet flavor, considered the finest Criollo grown, but other varieties may be included in this grade. Price 12 to 18 cents[b]
La Guaira Caracas	Venezuela	Criollo, lower priced than Puerto Cabello but very fine quality. Pale to deep brown color, with fine, full, sweet, and pronounced chocolate aroma. Price 10 cents[b]
Sanchez	West Indies	Forastero, dark brown to purple color, may be clayed; poor, unpleasant, bitter flavor. Bulk of this variety imported by the United States. Price 4.1 cents[b]
Trinidad	West Indies	Forastero, most uniform of all cacaos, red-brown color, slightly bitter taste, but fine chocolate flavor and second only to Ecuador cacao. Price 7.5 cents[b]

[a] Adopted from P. S. Lucas and I. A. Gould, *Mich. Quart. Bull.*, **22**, No 1, 12 (1939).
[b] Price per pound on New York market 1939.

In preparing cacao products for the market the manufacturer usually makes use of a blend of different cacaos. This is because of the consideration that the manufacturer must give to such details as economy, color standards, consumer's taste, and uniqueness of product. Table 206 gives the characteristics of seven popular cacaos. This list is by no means complete but includes the more important varieties.

(*g*) *Components of Cacao*

In addition to starch and other general matter, cacao is the source of these three constituents: cacao butter, theobromine, and cacao-purple (cacao-red).

Cacao butter is expressed from the cacao nibs. It is discussed in Volume II, Chapter XVIII. The fat, which forms from 52 to 56% of the kernel or nib, is a complicated mixture of glycerides.

TABLE 207

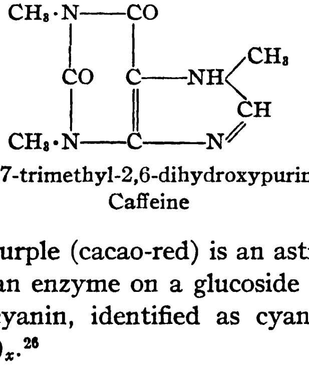
COMPOSITION OF CACAO BUTTER[a]

Analysis	%
Fully saturated glycerides (mixed palmitostearins)	2.5
Mono-oleo disaturated glycerides	77.0
Dioleo monosaturated glycerides	16.0
Triolein	4.0

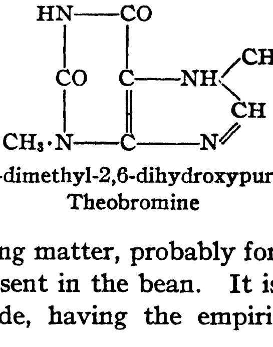
[a] C. H. Lea, *J. Soc. Chem. Ind.*, **48**, 41T (1929).

An important characteristic of this fat is that it is not likely to become rancid. Determination of the Reichert-Meissl number and the Iodine number of the cacao butter will show adulterations with such fats as coconut oil and palm-kernel oil. Adulteration with Borneo tallow (illipe butters) is difficult to detect unless specific tests, characteristic of the possible adulterant, are made.

Theobromine is an alkaloid found in cacao and its products. It is a stimulant although not as strong in its action as caffeine. Its formula is closely related to that of caffeine.[25]



1,3,7-trimethyl-2,6-dihydroxypurine
Caffeine



3,7-dimethyl-2,6-dihydroxypurine
Theobromine

Cacao-purple (cacao-red) is an astringent coloring matter, probably formed by the action of an enzyme on a glucoside originally present in the bean. It is considered an anthocyanin, identified as cyanidin-3-glucoside, having the empirical formula $(C_{34}H_{31}O_{13})_x$.[26]

6. Confectionery Flavors

The importance of flavors in confections cannot be overestimated. It is primarily because of flavor that the consumer selects one candy rather than another. It is difficult to define what is meant by the term flavor since it is really a combination of complicated sensations. Flavor not only includes the sensation of smelling and tasting but also the "mouth feel" of a food, *i. e.*, the physical effect of the food in the mouth. A coarse piece of milk chocolate will not have the same flavor as a finer-ground chocolate although the ingredients are the same. A tough marshmallow will have a different "eating quality" than a tender one.

---

[25] P. Schmidt, *Textbook of Organic Chemistry*. 2nd ed., Van Nostrand, New York, 1932, p. 344.
[26] Arthur W. Knapp, *Cacao Fermentation*. Bale, London, 1937, pp. 81, 96.

To produce a sour taste in candy the fruit acids, tartaric, citric, malic and, in special cases, acetic and lactic, are used. A salty taste is obtained by the use of the usual standby—sodium chloride. A bitter taste can be derived by the use of chocolate, after it has been toned down with milk solids, sugar, or other ingredients. The bitter principle extracted from grapefruit, known as naringin, may be used to enhance the piquant flavor of high-class confections, especially those pieces flavored with citrus fruit flavors. This substance, sold commercially as "Amerin," is so bitter that its taste can be detected in water at a concentration of 1:50,000.

The most popular flavor is vanilla. Vanilla flavor can be obtained from (*a*) vanilla beans, (*b*) vanilla extract, (*c*) vanillin, and (*d*) ethyl vanillin. Vanilla beans are the cured, full-grown but unripe fruit pods of a fragrant, reddish-brown and white parasitic orchid named *Vanilla planifolia.* The original home of the vanilla pods is Mexico, and the best grades of beans are still produced there. Vanilla beans of excellent quality and aroma, the "Bourbons" of commerce, are produced in the French islands of Madagascar, Comores, and Réunion, and the near-by British islands of Mauritius and Seychelles. Vanilla beans are also grown in the French West Indies (Guadeloupe and Basse Terre), Java, and Tahiti. A poor-quality bean, known as vanillons, grows wild in South America.

Vanilla resembles cacao in that it must be treated before it will produce its characteristic aroma. The vanilla pods are cured by scalding them and sun drying. During the sun drying a gradual fermentation takes place. A good, sound bean is long, straight, soft, and pliable, free from splits and blemishes, possesses a waxy or greasy dark chocolate color and is covered with an efflorescence ("givre") of white crystals of vanillin lying perpendicular to the furrows of the bean. The beans should have a smooth, vanilla aroma, free from any suggestion of mold, rot, or creosote.

Vanilla beans may be used alone after being cut up very fine and mixed directly with the product to be flavored. It may be first incorporated with sugar (1:5), ground very fine, and then used as vanilla sugar. In either case black specks of vanilla will be noticed in the flavored material. This is the only way of obtaining the natural vanilla flavor. Some of the flavoring principles of the vanilla bean can be extracted with alcohol. However, no extract gives as true a vanilla flavor as when the whole bean is used. Coumarin, heliotropin or tonquin bean extract are sometimes added to the vanilla extract to round off the vanilla flavor. One gallon of ordinary vanilla extract contains the extract from about $13^1/_2$ oz. of vanilla bean. A more concentrated extract, "Pound" vanilla, is also on the market. One pound of this extract contains the flavoring principles of 1 lb. of bean and may be used weight for weight in place of the bean. A still more concentrated product, an oleoresin (commercially called "Resinol") is available; 5 oz. of this extract are equivalent to 1 lb. of the bean.

One of the main components of vanilla flavor is vanillin. This compound was first isolated by Gabley in 1858. It occurs naturally in the vanilla pod, potato parings, asparagus, sugar beets, Siam benzoin, and certain blasams. Vanillin was synthesized in 1876 by Haarman and Tieman. It has the mouth-filling chemical name, *m*-methoxy-*p*-hydroxybenzaldehyde, also called methylprotocatechuic aldehyde.

Originally it was obtained by the oxidation of eugenol (70–80% in oil of cloves); more recently it has been obtained from the waste (lignin) of the wood-pulp industry. Since vanilla pods contain about 2% of vanillin, the flavoring strength of pod to vanillin is about 1:50. The flavor of vanillin alone does not duplicate the entire flavor of the vanilla bean. The odor of the natural product is more delicate and durable.

TABLE 208

PRICE AND FLAVOR COMPARISON OF THE THREE EXTRACTS[a]

Type of extract	Approximate comparative price per lb.	Approximate comparative price per flavor unit
Vanilla extract	135	1080
"Pound" vanilla	375	375
"Resinol"	1000	313

[a] "Flavors in Confectionery," T. Clendenning in *Mfg. Confectioner*, **20**, 31 (May, 1940).

Another synthetic vanilla flavor is "ethyl" vanillin. This is prepared from benzene. Its chemical structure is similar to that of vanillin with the exception that an ethyl group replaces a methyl group. Vanillin is C_6H_3—CHO—OCH_3—OH; ethyl vanillin is C_6H_3—CHO—OC_2H_5—OH. Ethyl vanillin is incorrectly named. The ethyl vanillin is, on the average, about 3$^1/_2$ times as strong as vanillin, besides having a finer flavor.

III. CANDY AS A FOOD

Although candy is included among the foods and the bulk of its ingredients are foods, unfortunately it is considered by many as a harmful article of diet. Candy is blamed for many evils of the flesh, including obesity, dental caries, high blood pressure, and constipation. Confectionery manufacturers, trying to counteract this unfavorable publicity, sometimes allow the pendulum to swing too far in the other direction. They want the world to know that their products are not only harmless but also very close to the perfect food, containing all necessary carbohydrates, fats, proteins, minerals, and vitamins needed to sustain life. Many "enriched" candies are produced so as to add to the usual popular appeal of candy the idea that it is now a more valuable foodstuff.

There is no doubt that these opinions are based on facts; the question is whether the conclusions arrived at are fair. It is true that candy contains very little roughage and is therefore completely digested and readily available for fuel purposes, but when eaten alone or in considerable amounts (100 g., approximately $^1/_4$ lb.) it depresses the secretion of the gastric juices and delays evacuation of the stomach; there is a resulting tendency of the stomach juices to absorb water from the lining of the stomach which, in turn, causes irritation.[27] The natural craving for "sweets" is increased by the palatability of the candy and encourages overeating with the accompanying evils which usually attend overindulgence in the eating of any other food. Eaten in moderation candy is a wholesome and highly nutritious food.

---

[27] R. J. Miller *et al.*, *Am. J. Physiol.*, **53**, 65 (1920).

In emergencies, a related product, chocolate, may be used, for a limited period, as a life-sustaining food. The first World War led to an increase in candy consumption, partly because of the food value soldiers had found in chocolate bars. In the present conflict, the United States Army supplies each soldier with an emergency kit that includes chocolate. (See Table 210.)

TABLE 209

VITAMIN VALUES OF A FEW IMPORTANT FOODSTUFFS[a]

Foodstuff	A[b]	B_1	C	D	B_2
Dairy butter	3800	0–39	0	100	0
Dried skim milk	0	110	0	0	640
Chocolate (sweetened)	0	10	0	Fair	Insufficient data
Chocolate (unsweetened)	0	10	0	100	Insufficient data
Cocoa	0	25	0	Fair	Insufficient data
Sugar	0	0	0	0	0
Cod-liver oil	85,000	0	0	5800	0
Almonds	Fair	100	0	0	Good
Brazil nuts	Fair	340	0	0	Good
Peanuts	26	220	0	0	200
English walnuts	830	130	0	0	Fair
Cocoa shell[c]	...	...	..	3500	...
Cocoa shell fat[c]	...	...	..	30,000	...

[a] Data are taken mainly from *Nutritional Charts*, published by H. J. Heinz & Co., Pittsburgh, Pa Additional information was also obtained from R. Whymper, "Vitamins," *Mfg. Confectioner*, 21 (May, 1941).

[b] A, B_1, C, and D are I. U. per 100 g. B_2 is expressed in Sherman-Bourquin Units per 100 g.

[c] U. S. Dept. Agr., *Misc. Pub.* 275 (1937).

TABLE 210

U. S. ARMY FIELD RATION D

Chocolate liquor	160 lbs.
Sucrose	160 lbs.
Milk, dry skim	70 lbs.
Cocoa butter	30 lbs.
Oat flour	20 lbs.
Vanillin	$^1/_2$ lbs.
Sufficient thiamine hydrochloride to give 600 I. U. of B_1 per 4-oz. bar.	
(Fuel value	600 cal. per 4-oz. bar)

Cocoa, a cacao product, is a healthful beverage that is more nutritious than either tea or coffee. Besides its food value, cocoa contains an alkaloid stimulant, theobromine, that is less stimulating than the caffeine of tea or coffee. Cocoa is not only less stimulating but also more sustaining.

When chocolate and candy are to be evaluated as foods it should be considered that they persist in their popularity because they are pleasurable delicacies, and that is how they should be judged.

Selected Bibliography

Austin, Alma H., *Romance of Candy.* Harper, New York and London, 1938.

Chatfield, C., and Adams, G., "Proximate Composition of American Food Materials," U. S. Dept. Agr., *Circ.* **549** (1940).

Daniel, E. P., and Munsell, H. E., "Vitamin Contents of Foods," U. S. Dept. Agr., *Misc. Pub.* **275** (1937).

Fiene, F., and Blumenthal, S., *Handbook of Food Manufacture.* Chemical Pub. Co., New York, 1938.

Fincke, H., *Handbuch der Kakaoerzeugnisse.* Springer, Berlin, 1936.

Hall, C. J. J. van, *Cacao.* Macmillan, London, 1932.

Hughes, Walter C., *Story of Candy.* Pamphlet, copyright 1930 by W. C. Hughes.

Jensen, Harold R., *Chemistry, Flavouring and Manufacturing of Chocolate Confectionery and Cocoa.* Blakiston, Philadelphia, and J. and A. Churchill, London, 1931.

Jordan, Stroud, *Confectionery Studies:* No. 1. *Confectionery Problems.* National Confectioners Assoc., Chicago, 1930. No. 2. *Confectionery Standards.* N. Y. Applied Sugar Laboratories, Inc., New York, 1933. No. 3. *Chocolate Evaluation.* N. Y. Applied Sugar Laboratories, Inc., New York, 1934.

Knapp, Arthur W., *Cacao Fermentation.* Bale, London, 1937.

Prescott, S. C , and Proctor, B E., *Food Technology.* McGraw-Hill, New York, 1937, Chap. XXI.

Whymper, Robert, *Cocoa and Chocolate.* Blakiston, Philadelphia, 1921.

Winton, Andrew L., *Microscopy of Vegetable Foods.* Wiley, New York, 1916.

Chapter XXII

COFFEE AND TEA

WILLIAM H. UKERS

New York, N. Y.

I. COFFEE

The word coffee comes from the Arabic *qahwah*, through the Turkish *kahveh*, being originally one of the names employed for wine in Arabic. This was the name given to the beverage. The berry from which the drink is made was called *bunn*.

1. Early History

Coffee was first mentioned in literature by Rhazes, an Arabian physician, about A.D. 900. It was first a food, then a wine, a medicine, and finally a beverage. Its use as a beverage dates back 700 years.

In the beginning the dried coffee berries were crushed and mixed with fat to form food balls. Then a wine was made from the raw beans and dried skins. The roasting of the beans began in the 13th century.

The coffee tree is indigenous to Ethiopia. From there its propagation spread to Arabia, India, Ceylon, Java, Martinique, Surinam, Brazil, the Philippines, and Mexico. Its most recent development has been in British East Africa and Indo-China.

The beverage was introduced from Arabia into Turkey, where the coffee house began in 1554; to Venice in 1615, to France in 1644, to England and to Vienna in 1650, and to North America in 1668. The first London coffee house was opened in 1652.

2. Production

The principal coffee-producing countries, according to their 1940–1941 exports, are Brazil, Colombia, Venezuela, British East Africa, Guatemala, El Salvador, Netherlands, Indies, Mexico, Haiti, Costa Rica, Ecuador, Nicaragua, Dominican Republic, British India, and Cuba (Fig. 78).

The world's annual production of coffee for the ten crop years from 1929–1939 averaged over 31,000,000 bags of 60 kg. (132.276 lbs.) each. Brazil averaged 22,410,600 bags yearly, or more than the production of all the rest of the world put together.

Brazil's coffee exports for the year 1941 amounted to 1,462,253,772 lbs. Of this total 1,296,941,180 lbs., or 88.7%, went to the United States. Ranking second among the world's shippers of coffee, Colombia exported in 1941, 385,122,235 lbs., the United States taking 93.4%. Shipping 96,555,527 lbs. in 1941, Venezuela ranks third among coffee-exporting countries, with the United States taking 66.5%. In 1940–1941 Kenya exported 19,572,112 lbs. of coffee, Tanganyika 34,787,536 lbs., Uganda 44,063,244 lbs., totaling 98,422,892 lbs. and making British East Africa the fourth largest coffee exporter. Ranking fifth as a coffee exporter, Guatemala's shipments for the year 1941 totaled 92,087,641 lbs. El Salvador exported 90,862,501 lbs. in 1941, thus ranking sixth among the world exporters of coffee. Normally ranking third among world coffee exporters, the Netherlands Indies shipped 145,528,944 lbs. in 1939, going principally to the European Continent and the United States; a small part to a few Far Eastern markets. Ranking eighth as a coffee exporter, Mexico shipped 61,410,323 lbs. of coffee in 1941, the United States taking almost all of it. In 1941 Haiti exported 61,251,063 lbs., thus holding ninth place as a world coffee exporter. Taking tenth place, Costa Rica, in 1941, shipped 51,658,275 lbs., 75% of which went to the United States. In 1941 Ecuador exported 26,115,780 lbs., of which 97% went to the United States. In 1941 Nicaragua exported 27,705,869 lbs., ranking eleventh among world exporters. The Dominican Republic exported

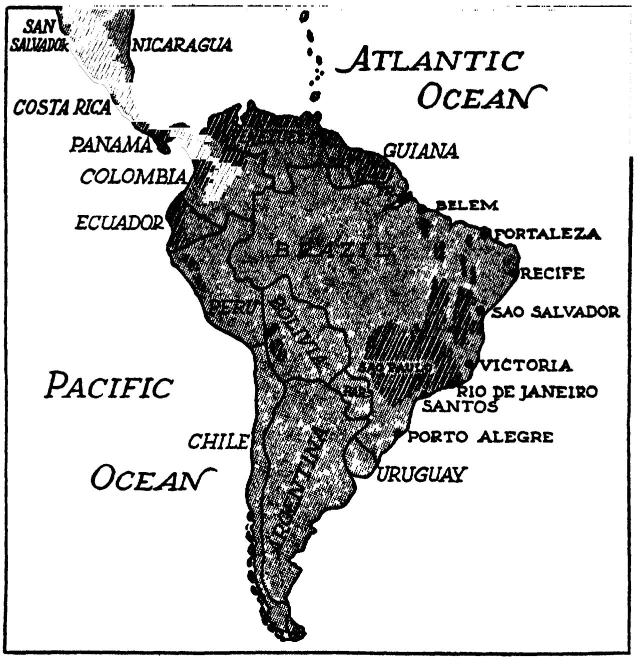


Fig. 78.—Coffee map of South and Central America showing distribution of the producing areas.

26,696,073 lbs. in 1941, of which 25,791,571 lbs., or 96.6%, went to the United States. Cuba's exports for 1941 were 10,046,097 lbs. The United States took 9,539,877 lbs. In 1941 Peru exported 6,900,045 lbs., of which the United States took 4,543,548 lbs., or 65.9%.

Before the crisis in the world coffee trade, Puerto Rico was an important coffee-exporting region, annually shipping from 20,000,000 to 50,000,000 lbs., practically all of which was destined for Spain and other European countries, and for Cuba, which absorbed approximately 40% of the Island's exports. The Island's shipments for 1940, only, amounted to 3,644,385 lbs., most of which was consumed in the United States Mainland. In 1941 Honduras exported 2,891,553 lbs., 92.4% of which went to the United States.

Other countries producing and exporting more than 1,000,000 lbs. of coffee annually are: Angola, Arabia, Belgian Congo, British India, Eritrea, Ethiopia, the Guianas, Hawaii, Ivory Coast, Liberia, Madagascar, New Caledonia, St. Thomas Island, and Princes Island.

3. Consumption

Arranged in the order of quantity, the principal coffee-consuming countries were, prior to World War II: United States, Germany, France, Sweden, Belgium, Netherlands, Denmark, Finland, Norway, Canada, Switzerland, Morocco, Egypt, and The United Kingdom.

The principal coffee-consuming countries based on prewar (1939) per capita figures were Denmark, 23.72 lbs.; Sweden, 19.69 lbs.; United States (1940), 15.48 lbs.; Finland, 15.09 lbs.; Norway, 15.97 lbs.; Belgium, 13.84 lbs.; Netherlands, 10.19 lbs.; France (1938), 9.79 lbs.; Switzerland, 11.59 lbs.; Germany (1938), 6.30 lbs.; Canada, 3.92 lbs.; South Africa, 3.3 lbs.; and the United Kingdom, 0.88 lb.

The United States imported 2,539,745,232 lbs. of coffee in 1940–1941. For many years it has led all other countries in the quantity of its imports. Its chief source of supply is Brazil which, in 1940–1941, provided 57.41% of our needs. Colombia supplied 22.31%; Venezuela, 3.57%; El Salvador, 3.27%; Guatemala, 3.24%; Mexico 2.36%; Haiti, 1.55%; Costa Rica, 1.12%; Ecuador, 1.01%; and other countries, 4.16%. Our per capita consumption in 1941 was 16.97 lbs., preliminary figures.

4. Botany

The coffee plant most cultivated for its berries is *Coffea arabica,* although other species are not infrequently met in the trade, notably *liberica* and *robusta.* The *arabica* is an evergreen shrub that reaches a height of 14 to 20 ft., but usually is kept down to 6 ft. It bears fruit, leaf, and blossom at the same time.

The leaves of the coffee tree, 3 to 6 in. in length, are lance-shaped, being borne in pairs, opposite each other. They are dark green on the upper surface and light green underneath. The coffee berries are dark green at first, changing as they mature to yellow, red, and finally deep crimson or cranberry color. Beneath the skin of the berry, or coffee in the "cherry," is a mucilaginous saccharine pulp, enveloping the parchment covering which encloses the green coffee beans, usually a pair of oval plano-convex seeds, though sometimes there is but one seed, called from its shape peaberry or male berry. The green beans are wrapped in a delicate, semitransparent, closely adhering jacket called the silver skin.

The small white blossoms are not unlike those of the jasmine or orange in form and scent. They are tubular, the tube of the corolla dividing into five white segments, though the number of petals is not at all constant even for flowers of the same tree. They last about three days. In countries where the coffee estates are near the coast their rich fragrance may be detected by incoming voyagers three miles from land.

5. Chemical Aspects

Generally speaking, the trade and the consumer are concerned chiefly with those factors which enter into coffee goodness. These are the caffeine content and the caffeol. Caffeine supplies the principal stimulant. It increases the capacity for muscular and mental work without harmful reaction. The caffeol supplies the flavor and the aroma—that indescribable oriental fragrance that woos us through the nostrils, forming one of the principal elements that make up the lure of coffee. There are several other components, including certain innocuous so-called caffetannic acids, which, in combination with the caffeol, give the beverage its rare gustatory appeal.

In the roasting of green coffee, part of the original caffeine content is lost by sublimation (vaporizing), and caffeol is formed. Chemists recognize two groups of components which are formed during roasting and are soluble in water—heavy extractives and light aromatic materials.

The heavy extractives include caffeine, mineral matter, proteins, caramel, and sugars, "caffetannic acid," and various organic materials. Some fat will also be found in the average coffee brew, melted from the bean by the heated water and carried along with the solution. The light extractives are collectively known as caffeol.

Caffeine has a slightly bitter taste. It furnishes the stimulation for which coffee is generally consumed. The average caffeine content of *C. arabica*, green, is 1.5%; when roasted about the same. The caffeine content of a cup of coffee is 1.5 grains.

The mineral matter, together with certain decomposition and hydrolysis products of crude fiber and chlorogenic acid, contributes toward the astringency or bitterness of the cup. The proteins are present in such small quantity that their only role is to raise somewhat the almost negligible food value of a coffee infusion. The body, or what might be called the licorice-like character of coffee, is due to the presence of bodies of a glucosidic nature and to caramel. The degree to which a coffee is sweet tasting or not is, of course, dependent upon its other characteristics, but probably varies directly with the reducing sugar content.

The term "caffetannic acid" is a misnomer, for the substances called by this name are in all probability mainly coffalic and chlorogenic acids, niether of which is a true tannin. They evince but few of the characteristic reactions of tannic acid. Some neutral coffees will show as high a "caffetannic acid" content as other coffees of acid character. Careful chemical analysis has shown that the actual acidities of some East Indian coffees vary from 0.013 to 0.033%. These figures may be taken as reliable examples of the true acid content of coffee, and, though they seem very low, it is not at all incomprehensible that the acids they indicate produce the acidity in a cup of coffee. They probably are mainly volatile organic acids together with other acidic-natured products of roasting.

TABLE 211

COMPARATIVE ANALYSES OF COFFEES FROM FOUR DIFFERENT COUNTRIES[a]

Analysis	Santos		Padang		Guatemala		Mocha	
	Green	Roasted	Green	Roasted	Green	Roasted	Green	Roasted
Moisture								
April 20	8.75	3.75	8.78	2.72	9.59	3.40	9.06	3.36
Sept. 20	8.12	6.45	8.05	6.03	8.68	6.92	8.15	7.10
Ash	4.41	4.49	4.23	4.70	3.93	4.48	4.20	4.43
Oil	12.96	13.76	12.28	13.33	12.42	13.07	14.04	14.18
Caffeine	1.87	1.81	1.56	1.47	1.26	1.22	1.31	1.28
Crude fiber	20.70	14.75	21.92	14.95	22.23	15.23	22.46	15.41
Protein	9.50	12.93	12.62	14.75	10.43	11.69	8.56	9.57
Water extract	31.11	30.30	30.83	30.21	31.04	30.47	31.27	30.44

[a] Adapted from Trigg's analytical studies of coffee in W. H. Ukers, *All About Coffee*. Tea and Coffee Trade Journal Co., New York, 1922.

We know that very small quantities of acid are readily detected in fruit juices and beer, and that variation in their percentages is quickly noticed, while the neutralization of this small amount of acidity leaves an insipid drink. Hence it seems likely that this small acid content gives to the coffee brew its essential acidity. A few minor experiments on neutralization have resulted in the production of a very flat beverage by thus treating a coffee infusion. The acidity of certain coffees apparently should be attributed to such compounds rather than to the misnamed caffetannic acid. This point may be illustrated by putting a small pinch of the weakly alkaline baking soda ($NaHCO_3$) into a cup of coffee and noting the difference that it makes in flavor.

The light aromatic materials and other substances that are steam-distillable (*i. e.*, which are driven off when coffee is concentrated by boiling) are important factors in determining the individuality of coffees. These compounds (caffeol) vary greatly in the percentages present in different coffees, and thus are largely responsible for our ability to distinguish coffees in the cup. It is these compounds that supply the pleasing aromatic and appetizing odor to coffee.

Like all good things in life, the drinking of coffee may be abused. Indeed, those having an idiosyncratic susceptibility to alkaloids should be temperate in the use of tea, coffee, or cocoa.

A brief summary of available information on the pharmacology of coffee indicates that it should be used in moderation, particularly by children, the permissible quantity for adults varying with the individual, his constitution, mode of living, etc., and ascertainable only through personal observation.

TABLE 212

WORKS PUBLISHED ON THE AROMATIC PRINCIPLES OF COFFEE[a]

Investigator	Reference	Components
O. Bernheimer[b]	*Monatsh.*, **1**, 456 (1880)	A methyl derivative of saligenin, caffeine, higher fatty acids, acetic acid, hydroquinone, methylamine, pyrrol, acetone
A. Monari and L. Scoccianti[b]	*Annali Chimice Fermacolaia*, **1**, 70 (1895)	Pyridine
H. Jaeckle[b]	*Z. Untersuch. Nahr. u. Genussm.*, **1**, 457 (1898)	Furfural, caffeine, pyridine, ammonia, trimethylamine, acetic and formic acids, acetone
E. Erdmann	*Ber.*, **35**, 1946 (1902)	An isovaleric acid in large amounts, a little acetic acid, phenols, furfuryl alcohol, and a fraction b. p. 93° at 13 mm. containing nitrogen and having the odor of coffee (in extremely small amounts)
G. Bertrand and G. Weismuller	*Compt. rend.*, **157**, 212 (1913)	Pyridine 200–250 mg./kg. coffee
Sayre	*Bull. Pharm.*, **30**, 276 (1906)	Pyridine
R. E. Sethness	*Tea Coffee Trade J.*, **46**, 570 (1924)	Methyl ether of saligenin claimed to be principal constituent of coffee oil
H. Schmalfuss	*Biochem. Z.*, **216**, 330 (1929)	Diacetyl

[a] From W. H. Ukers, *All About Coffee.* 2nd ed., Tea and Coffee Trade Journal Co., New York, 1935.

[b] Analyzed condensed roaster gases.

Used in moderation, coffee has invariably proved a valuable stimulant, increasing personal efficiency in mental and physical labor. Its action in the alimentary regime is that of an adjuvant food, aiding digestion, favoring increased flow of the digestive juices, promoting intestinal peristalsis, and not tanning any part of the digestive organs. It reacts on the kidneys as a diuretic, and increases the excretion of uric acid, which, however, is not to be taken as evidence that it is harmful in gout. Coffee has been indicated as a specific for various diseases, its functions therein being the raising and sustaining of low vitalities. Its effect upon longevity is virtually nil. A small proportion of humans who are very nervous may find coffee undesirable, but sensible consumption of coffee by the average, normal, nonneurasthenic person will not prove harmful but beneficial.

Prescott[1] has summarized the effects of coffee as follows:

"It may be stated that, after weighing the evidence, a dispassionate evaluation of the data so comprehensively surveyed has led to no alarming conclusions that coffee is an injurious beverage for the great mass of human beings, but on the contrary that the history of human experience, as well as the results of scientific experimentation, points to the fact that coffee is a beverage which, properly prepared and rightly used, gives comfort and inspiration, augments mental and physical activity, and may be regarded as the servant rather than the destroyer of civilization."

---

[1] S. C. Prescott, *All About Coffee.* Tea and Coffee Trade Journal Co., New York, 1935, p. 314.

6. Cultivation

Generally speaking, the most suitable climate for coffee is a temperate one within the tropics. *Robusta* and *liberica* do best in regions from sea level to 3000 ft. *Arabica* flourishes better at higher levels up to the 6000-ft. frost line. Usually the trees are grown from seed first planted in nursery beds and when about one year old are transferred to prepared plantation grounds, where they are set out in shallow holes 8 to 12 ft. apart. They bear full crops in 6 years.

The coffee fruit ripens about 6 to 7 months after the tree has flowered or blossomed, and becomes a deep purplish-crimson color. It is then ready for picking. The ripening season varies throughout the world, according to climate and altitude. In the state of São Paulo, Brazil, the harvesting season lasts from May to September, while in Java, where three crops are produced annually, harvesting is almost a continuous process throughout the year. In Colombia, the harvesting seasons are March to June and October to January. In Guatemala the crops are gathered from October through December; in Venezuela, from November through March. In Mexico the coffee is harvested from November to January; in Haiti the harvest extends from November to March; in Arabia, from September to March; in Ethiopia, from September through November. In Uganda, Africa, there are two main crops, one ripening in March and the other in September, and picking is carried on during practically every month except December and January. In India the fruit is ready for harvesting from October to January.

7. Preparation

Coffee is prepared for the market by the "dry" and the "wet" methods. In the former the berries are sun-dried and cleaned by hand or machines in the dried state. In the latter they are fermented and pulped in water and dried in the sun or by artificial means before being machine-cleaned.

The cleaned beans are transported to the nearest seaport town where, before shipment overseas, they are not infrequently graded and tested by buyers representing the larger importers in the principal consuming countries. Arrived in the consuming country the green beans are roasted by coal or gas, packed, and distributed to the consumer through the recognized channels.

8. Roasting and Grinding

(*a*) *Roasting*

The modern way to roast coffee is in revolving, perforated metal cylinders, heated by gas fuel. The roaster man, or coffee chef, is the first and most important cook necessary to a good cup of coffee.

A study of the chemistry of roasted coffee discloses that in the roasting process the beans swell up by the liberation of gases, the aromatic oils are developed or cooked and made ready for solution in water when the cells are broken up by grinding.

The roasting operation is preceded by separating, milling, and mixing. Where the coffees have not been graded and cleaned before delivery to the roasting plant, they must be run through a separator for grading closely as regards the size of the beans, and particularly for the separation of round beans, or peaberry. Then they must be milled and mixed. This operation may be performed in special machines designed for these purposes, or the regular roasting cylinder may be utilized.

A typical roasting machine employs an open perforated metal cylinder, the inside being arranged with reverse spiral flanges which mix the coffee, while the cylinder revolves over the fire. It is fitted with a front head opening to receive the green coffee, and through this, when turned to the lower position, the finished product is discharged into the cooler box.

Modern coffee-roasting machines provide for easy control of the heat (from coal, coke, or gas fuel), for constantly mixing the coffee in such a manner that the heat is transmitted uniformly to the entire batch, for carrying away all steam and smoke rapidly, for easy testing of the progress of the roast, and for immediate discharge when desired. The operator's problem, therefore, is the regulation of the heat and deciding just when the desired roasting has been accomplished. The transfer of heat in coffee roasting is discussed by Backer.[2]

If all coffees were alike, roasting would soon be almost automatic. In some plants most of the work is one uniform grade or blend; but coffees which vary greatly in moisture content, in flinty or spongy nature, and in various other characteristics, will puzzle the operator until he establishes a personal acquaintance with them in various combinations in repeated roasting operations. The roaster man, therefore, must be able to observe closely, to draw sensible conclusions, and to remember what he learns. Roasting coffee is work of a sort which anybody can do, which a few people can do really well, and no one so well but that further improvement is possible.

Because the roasting principles vary in different green coffees, trained study and a nice science in timing the roast and manipulating the fire are necessary to a perfect development of aroma and flavor. There is no absolute standard of what the best roasting results are. Some dealers want the coffee beans swelled up to the bursting point, while others would object to so showy a development. Some care nothing at all about appearance as compared with cup value, while others insist on a bright style even at some sacrifice of quality. Business judgment must decide what goods can be sold most profitably.

Coffee roasting requires a temperature of about 420° F. A slow roast is favored by some roaster men; others argue that this bakes the coffee and does not give full development. The quicker the roast, the better the coffee.

---

[2] L. H. Backer, *Tea Coffee Trade J.*, **70**, 372 (1936).

So there is no universal rule for the degree to which coffee should be roasted. The average time in the United States is 15 to 30 min., depending on the fuel and machine employed. (The Germans have *quick roasters* that do it in $3^1/_2$ to 10 min.) The trade knows these different roasts: light, cinnamon, medium, high, city, full city, French, and Italian. The city roast is a dark bean, while full city is a few degrees darker. In the French roast, the bean is cooked until the natural oil appears on the surface, and in the Italian it is roasted to the point of actual carbonization, so that it can be easily powdered. Germany likes a roast similar to the French type, while Scandinavia prefers the high Italian roast.

In the United States, the lighter roast is favored on the Pacific coast; the darkest, in the South; and a medium-colored roast, in the eastern states. The cinnamon roast is most favored by the trade in Boston.

Coffee loses weight in roasting; the loss varying, depending upon the kind of bean, its age, and the style of roast. The average loss is about 16%. It has been estimated that 100 lbs. of coffee in the cherry produce 25 lbs. in the parchment; that 100 lbs. in the parchment produce 84 lbs. of cleaned coffee; that 100 lbs. of cleaned coffee produce 84 lbs. of roasted.

After the coffee has been in the roasting cylinder for a short time, the color of the bean becomes a yellowish brown, which gradually deepens as it cooks. Likewise as the beans become heated, they shrivel up until about half done, or at the developing point. At this stage, they begin to swell, and then pop open, increasing 50% in bulk. That is when the experienced roaster man turns on all the heat he can command, to finish the roasting as quickly as possible. The roast is considered done when the bean cracks easily between the fingers. Some roaster men use their teeth, others the palm of the hand and a coffee trier.

At frequent intervals, he thrusts his "trier"—an instrument shaped somewhat like an elongated spoon—into the cylinder, and takes out a sample of coffee to compare with his type sample. When the coffee is done, he shuts off the heat and checks the cooking by reducing the temperature of the coffee and of the cylinder as quickly as can be done. In the wet roast method he will spray the coffee, as the cylinder is still revolving, with 3 to 4 qts. of water to every 130 lbs. of coffee. In the dry method he depends altogether upon his cooling apparatus.

Roasters generally are not in favor of the excessive watering of coffee in and after the roasting process for the purpose of reducing shrinkage. "Heading" the coffee, or checking the roast before turning it out of the roasting cylinder, is another matter and is considered legitimate.

Finishing whole-bean roasted coffee by giving it a friction polish when it is still moist, using a glaze solution, or water only, is a practice not harmful if the proper solutions are employed. A machine has been developed for finishing or glazing. Coatings of sugar and eggs, glucose, mustard oil, and chicory are sometimes employed, but their use must be stated on the label.

The cooling and stoning operation which follows the roasting requires efficient apparatus.

Generally speaking, the process is to dump the roast into a metal car having a perforated false bottom, to which is attached a powerful exhaust fan that sucks the heat out of the coffee. The stoner has for its function the removal of stones and other foreign matter which the green-coffee operations have failed to get rid of. Usually the coffee beans are carried up a pipe by a regulated air current which is strong enough to raise the coffee but not the stones, which remain at the bottom of the stoner boot, whence they are dumped at intervals into a pan underneath.

(*b*) *Grinding*

After cooling and stoning, unless it is to be polished or glazed, the coffee is ready for grinding and packing, if it has been blended in the green state. Otherwise, the next step will be to mix the different varieties before grinding, although some packers blend the different kinds after they have been ground. To mix whole-bean roasted coffee without marring its appearance is rather difficult, and there is no regular machine for such work.

Unless the coffee is to be sold in the bean, it is sent to the grinding and packing department, to be further prepared for the consumer. Since the Federal Food Law has been in effect, the public has gained confidence in ground and bean coffee in packages; and today a large part of the coffee consumed in the United States is sold in 1- and 2-lb. cartons and cans, already blended and ready for brewing.

A progressive coffee-packing house may have three different styles of grinding machines; one called the granulator for turning out the so-called steel-cut coffee; the second, a pulverizer for making a really fine grind; and the third, a grinding mill for general factory work and producing a medium-ground coffee.

Commercial coffee-grinding machines are alike in principle in all countries, the beans being crushed or broken between toothed or corrugated metal or stone members, one revolving and the other being stationary. While all grinding machines are alike in principle, they may vary in capacity and design. The average granulator will turn out about 500 lbs. of steel-cut coffee in an hour; the pulverizer, 75 to 100 lbs.; and the average grinding mill, 500 to 600 lbs. Some types of grinding machines have chaff-removing attachments to extract, by air suction, the chaff from the coffee as it is being ground.

A large number of trade terms for designating different grinds of coffee are used in the United States, some of them meaning the same thing, while similar names are sometimes contradictory. Some years back a canvass of the leading American coffee packers disclosed that there were 15 terms in use, and that there were 34 different meanings attached to them. For the term *fine* there were five different definitions; *medium* had five; *coarse*, seven; *pulverized*, four; *steel-cut*, seven; *ground*, two; *powdered*, one; *percolator*, two; *steel-cut-chaff-removed*, one; *Turkish ground*, one; while *granulated*, *Greek ground*, *extra fine*, *standard*, and *regular* were not defined.

The term steel-cut is generally understood to mean that in the grinding process the chaff has been removed and an approximate uniformity of granules has been obtained by sifting. The term does not necessarily mean that the grinding mills have steel burrs. In fact, most firms employ burrs made of cast iron or of a composition metal known as *burr metal,* because of its combined hardness and toughness.

The steel-cut "idea" is another of those sophistries for which American advertising methods have been largely responsible in the development of the package-coffee business in the United States. The term steel-cut lost all its value as an advertising catchword for the original user when every other dealer began to use it, no matter how the ground coffee was produced. When the public has been taught that coffee should be steel-cut, it is hard to sell it ground coffee unless it is called steel-cut; although a truer instructor of the consumer would have caused him to insist on buying whole-bean coffee to be ground at home.

Steel-cut coffee—that is, a medium-ground coffee with the chaff blown out—does not compare in cup test with coffee that has been more scientifically ground and not given the chaff-removal treatment that is largely associated in the public mind with the idea of the steel-cut process.

According to the results of the trade canvass previously referred to, it would appear that the terms most suited to convey the right idea of the different grades of grinding, and likely to be acceptable to the greatest number, would be *coarse* (for boiling and including all the coarser grades); *medium* (for coffee made in the ordinary pot, including the so-called steel-cut); *fine* (like granulated sugar, and used for percolators); *very fine* (like corn meal, and used for drip or filtration methods); *pulverized,* for glass vacuum type; *powdered* (like flour, and used for Turkish coffee).

Coffee begins to lose its strength immediately after roasting, the rate of loss increasing rapidly after grinding. In a test carried out by a Michigan coffee packer, it was claimed that a mixture of a very fine with a coarse grind gave the best results in the cup. It was also claimed that coarse-ground coffee lost its strength more rapidly than the medium ground; while the latter deteriorated more quickly than a fine grind; and so on, down the scale. His conclusions were that the most satisfactory grind for putting into packages, that were likely to stand for some time before being consumed, was a mixture consisting of about 90% finely ground coffee and 10% coarse. His theory is that the fine grind supplies sufficiently high body extraction; the coarse, the needful flavor and aroma. On this irregular grind a United States patent (No. 14,520) was granted, in which the inventor claimed that the 90% of fine eliminates the interstices—that allow too free ventilation in a coarse-ground coffee—and consequently prevents the loss of the highly volatile constituents of the 10% of coarse-ground particles. However, both the trade and the best scientific opinion believe that the finer the grind the quicker the staling.

As long as there continues a consumer demand for ground package coffee, there will be found manufacturers willing to supply it, despite all the well-turned arguments in favor of grinding at home or in the shop at the time of purchase.

There are factory mills to be had in which coffee may be reduced to the desired fineness at one passage through a pair of metal-disk grinding plates, which are capable of producing 500 lbs. an hour of finely ground coffee such as will pass completely through a square-mesh sieve having $^3/_{64}$-in. clear openings. These mills can be made to produce as much as 1000 to 1500 lbs. of finely ground coffee an hour. For retail distributors there are many excellent counter mills that render efficient service in grinding whole-bean loose or package coffee for the housewife while she waits.

9. Marketing

Roasted coffee is sold at wholesale in the United States chiefly by about 5000 wholesale grocers and by roasters who make a specialty of preparing the green coffee for consumption and who feature either bulk or trade-marked package goods. It is sold at retail in the United States through seven distinct channels of trade: independent retail grocers, chain stores, mail-order houses, house-to-house wagon-route distributors, specialty tea and coffee stores, department stores, and drug stores.

The principal coffee-trading markets of the world are in New York, Le Havre, Hamburg, and Antwerp. There are both spot and future markets in New York. In the former, actual coffee is handled in street sales. In the latter, the trading is not concerned with actual coffee but with the purchase or sale of contracts for future delivery of coffee that may still be on the trees in the producing countries. These operations, which take place on the coffee Exchange, are either in the nature of ordinary speculation on margin or for the legitimate purpose of effecting hedges against holdings or short sales of actual coffees.

Under the United States import regulations it is illegal to import into this country a coffee that grades below a No. 8 Exchange type, which generally contains a large proportion of sour or damaged beans, known in the trade as "black jack," or damaged coffee, as found in "skimmings." Black jack is a term applied to coffee that has turned black during the process of curing, or in the hold of a ship during transportation; or it may be due to a blighting disease.

Another ruling is intended to prevent the sale of artificially "sweated" coffee, which has been submitted to a steaming process to give the beans an extra-brown appearance. Up to the time that the Pure Food and Drugs Act went into effect, artificial sweating was resorted to by some coffee firms; and out of that practice grew a suit that resulted in a federal court decision sustaining the Pure Food Act, and classifying the practice as adulteration and misbranding.

The act also is intended to prevent the sale of coffees under trade names that do not properly belong to them. For example, only *arabica* coffees grown on the island of Java can properly be labeled and sold as Javas; coffees from Sumatra, Timor, etc., must be sold under their respective names. Food Inspection Decision No. 82, which limited the use of the term Java to coffee grown on the island of Java, was sustained in a service and regulatory announcement issued in January, 1916. Likewise the name Mocha may be used only for coffees of Arabia. Before the Pure Food Law was enacted, it was frequently the custom to mix Bourbon Santos with Mocha and to sell the blend as Mocha. Also, Abyssinian coffees were generally known in the trade as Longberry Mocha, or just straight Mocha; and Sumatra growths were practically always sold as Javas. Traders used the names of Mocha and Java because of the high value placed upon these coffees by consumers, who, before Brazil dominated the market, had practically no other names for coffee.

Another important ruling, concerning coffee buyers and sellers, prohibits the importation of green coffees coated with lead chromate, Prussian blue, and other substances, to give the beans a more stylish appearance than they have normally. Such "polished" coffees find great favor in European markets, but are denied admittance in the United States.

The Board of Food and Drug Inspection decided in 1910 against a trade custom that had prevailed until then of calling Minas coffee *Santos* when shipped through Santos instead of Rio.

For years a practice obtained of rebagging certain Central American growths in New York. In this way Bucaramangas frequently were transformed into Bogotas, Rios became Santos, Bahias and Victorias were sold as Rios, and the misbranding of peaberry was quite common. A celebrated case grew out of an attempt by a New York coffee importer and broker to continue one of these practices after the Pure Food Act made it a criminal offense.

After an exchange of views with the United States Board of Food and Drug Inspection, the New York Coffee Exchange decided that, after June 1, 1912, it would abolish all grades of coffee under the Exchange type No. 8.

The practice in Holland of grading Santos coffees by selecting beans most like Java beans, and polishing and coloring them to add verisimilitude—known as "manipulated Java"—became such a nuisance in 1912 that United States consuls refused to certify invoices to the United States unless accompanied by a declaration that the produce was "pure java, neither mixed with other kinds nor counterfeited."

The United States Bureau of Chemistry ruled in February, 1921, that *Coffea robusta* could not be sold as Java coffee, or under any form of labeling which tended either directly or indirectly to create the impression that it was *C. arabica*, so long and favorably known as Java coffee. This was in line with the Department of Agriculture's previous definition that coffee was the seed of *C. arabica* or *C. liberica*, and that Java coffee was *C. arabica* from Java. *C. robusta* was barred from deliveries on the New York Coffee Exchange in 1912, but since 1925 the rules have permitted the delivery of washed *robusta*, grading below No. 7, on contract "A."

More than a hundred different kinds of coffee are bought and sold in the United States. They are divided into two general groups, Brazils and Milds. Brazils comprise those coffees grown in São Paulo, Minas Geraes, Rio de Janeiro, Bahia, Parana, and other Brazilian states. The Milds include all coffees grown elsewhere. Brazils make up about 60% of the world's total consumption.

10. Trade Characteristics

Brazil coffees are classified into four great groups, which bear the names of the ports through which they are exported: Santos, Rio, Victoria, and Bahia. Santos coffee is grown principally in the State of São Paulo; Rio, in the state of Rio de Janeiro, and the state of Minas Geraes; Victoria, in the state of Espirito Santo; and

Bahia in the state of Bahia. All of these groups are further subdivided according to their bean characteristics and the districts in which they are produced. Parana is a comparatively new producing state. Its port is Paranagua.

Bourbon Santos is the best of the Brazil growths. It is a small bean resembling Mocha. It is used with any high-priced coffee to reduce the blend. The flat bean Santos may be used straight or in combination with all milds. Rios have a pungent flavor and aroma and are useful for popular-priced package blends. Minas coffees are similar to Santos.

Mexicans are mellow, rich in body, and of fine acidity. The best-known districts are Coatepec, Huatusco, and Orizaba.

The best-known Guatemalas are Cobans and Antiguas. They have an aromatic cup and are fine blenders. Salvadors are mostly inferior to Guatemalas. Nicaragua's washed coffees are fine roasters and acid in the cup. The high-altitude coffees of Costa Rica are rich in body, of fine, mild flavor, and make superior blenders. Cuban coffees resemble in flavor the fine washed coffees of Santo Domingo.

Haiti's coffees are of average quality. Santo Domingo coffees are of good body and fair flavor. The Blue Mountain Jamaicas are fancy roasters, rich, full, and mellow in the cup.

Puerto Rico coffees are fancy roasters having a peculiar flavor similar to washed Caracas.

The best-known Colombians are Medellins (handsome roasters; fine flavor and body), Manizales (similar to Medellins but not so acid), Armenias, Bogotas, and Bucaramangas, the fancies among which compare favorably with fine Javas and Sumatras.

Venezuelan coffees range next to Santos in quality and price. Washed Caracas are best in roast and cup. Meridas are the best of the Maracaibos, having a delicate flavor prized by experts.

Mocha coffees have a unique, acid character, and a heavy body which make them useful for blending with fancy mild washed types.

In recent years a high-grade mild coffee grown in Kenya Colony, East Africa, has been received with favor by consumers in Europe and America.

Of the Indian coffees Malabars are best. Mysore growths come next. Plantation Ceylons are stylish roasters, rich and flavory in the cup.

The best Java coffees come from the Preanger, Cheribon, Buitenzorg, and Batavia districts. The Sumatra growths are better. The finest coffees in the world come from the Mandheling and Ankola districts.

Among the Ethiopian growths the Longberry Harar resembles Mocha in the cup.

Old crop Kona growths from Hawaii are of fine flavor and blend well with any high-grade mild coffee.

For a full discussion of the trade values and cup characteristics of the principal kinds of coffees grown in the world, buyers are referred to the trade publications.[3]

---

[3] W. H. Ukers, *All About Coffee.* 2nd ed., Tea and Coffee Trade Journal Co., New York, 1935; *Coffee Merchandising.* Tea and Coffee Trade Journal Co., New York, 1930.

11. Coffee Making

The correct preparation of coffee requires that the beans be freshly roasted and ground, the grind varying according to the type of brewing device used. The trend is toward the increasing use of coffee makers embodying the drip or filtration principle.

Prescott[4] points out the following: roasted coffee retains its characteristic freshness for comparatively few days. When exposed to air it gradually acquires a dullness or a flatness of flavor, and eventually a staleness in odor and taste that rob it of its pleasing effect on the sense organs of smell and taste. The exact changes which take place are unknown, although one marked feature is the reduction in the amount of carbon dioxide that is held in occluded or absorbed condition within the roasted bean. This loss seems in part at least to be a gaseous interchange between carbon dioxide and oxygen, and in coffee exposed freely to air the change in taste is begun within 2 or 3 days after roasting, and produces changes in flavor which may be readily detected by the fourth or fifth day. If the coffee is in an hermetically sealed package, these marked changes do not occur until the package is opened and air exposure takes place.

Three methods of brewing coffee are in common use, and may be briefly considered:

(*1*) The old-fashioned method of preparing coffee by boiling.

(*2*) The use of the so-called percolators, in which the coffee grounds are repeatedly sprayed by successive portions of water and then of the infusion itself as it results from the long-continued action.

(*3*) The use of devices of various types which produce filtered or "drip" coffees; *i. e.*, prepared by the single, or sometimes repeated, passage of hot water through the mass of ground coffee, the infusion passing into a receptacle below.

In the first and second of these methods the temperature is often too high and the time of treatment too long for best results. The volatile components may be lost and the coffee become strong not because of increased caffeine content but because of the larger quantity of dissolved coloring matters, woody extract, and other slowly soluble ingredients. The third method lends itself to a definite control of temperature, time of contact of water and coffee, and retention of the desired substances and exclusion of those less desirable.

Prescott[5] believes the best results will be obtained with freshly roasted coffee, infused at temperatures 185 to 195° F. for not over 2 min. in a glass, porcelain, or vitrified container, and immediately filtered from the grounds.

---

[4] S. C. Prescott, *All About Coffee.* 2nd ed., Tea and Coffee Trade Journal Co., New York, 1935, p. 653.

[5] S. C. Prescott, special report to the author.

12. Concentrates and Substitutes

The manufacture of prepared, or refined, coffees has become an important business in the United States and Europe. Prepared coffees can be divided into three general groups: treated coffees, from which the caffeine has been removed to some degree; dry coffee extracts (soluble coffee), which are readily dissolved in a cup of hot or cold water; and liquid coffee concentrate, to which water is added for making the coffee beverage.

A Japanese chemist, Dr. Sartori Kato, brought a soluble tea to Chicago in 1899. It was not a success but he later produced a soluble coffee which was first sold to the public at the Pan-American Exposition in 1901. Dr. Sartori was granted several United States patents on coffee concentrates, or soluble coffees.

G. Washington, who was born in Belgium of English parents, invented, about 1906 in Guatemala City, a soluble coffee later to bear his name, but called Red E when first marketed in 1909. In Europe a similar product was called *Belna*. There followed in the United States many rival products, variously called soluble, instant, or minute coffees. In the first World War soluble coffee was added to the reserve ration of our troops overseas. After trying to put it up in sticks, tablets, and capsules, it was determined that the best method was to pack it in envelopes. The general method for producing soluble coffee is to extract the drinking properties from ground roasted coffee by means of water, and to evaporate the resulting liquid until only the powder is left.

Decaffeinated, or caffeine-free, coffees started with the introduction into America of *Dekafa* by Ludwig Roselius of Bremen. Later it became *Dekofa*, and then *Kaffee Hag*. Rights under the original German patent were sold in France, where the product became *Sanka*, and in England where it was known as *Life Belt*. Several improvements on the process were subsequently patented. The most common practice is to make the green beans soft by steaming under pressure and then to apply benzol, chloroform, or alcohol to the softened coffee to dissolve and extract the caffeine. Afterward, the extracting solvents are driven out of the coffee by resteaming. However, chemists have not yet been able to expel all the caffeine in treating coffee commercially, the best efforts resulting in from 0.3 to 0.07% remaining. After treatment, the coffee beans are roasted, packed, and sold like ordinary coffee.

In 1919, Trigg and Hamor were granted a patent in the United States for making an aromatized coffee extract. In this process, the caffeol of the coffee is volatilized and then brought into contact with an absorbing medium such as is used in the extraction of perfumes. The absorbing medium is then treated with a solvent of the caffeol, and the solution is separated from the petrolatum. Then the coffee solution is concentrated to an extract by evaporation; after which the extract and the caffeol are combined into a soluble coffee. Five additional patents were granted on this same process in 1921.

Since then a number of liquid coffee brands have appeared on the market, with indifferent success. Difficulty has been experienced in producing a liquid product that compares with regular coffee in cup quality or that will retain its flavor for any length of time. In 1939, Dr. David Isrin, a New York chemist who was formerly a perfume manufacturer in Russia and Japan, produced a liquid coffee concentrate which he claimed required no preservative and did not oxidize or deteriorate when used as a flavoring agent.

In 1934, the Coffee Board of Kenya, East Africa, purchased an interest in the manufacture and distribution of a new type of coffee tablet (*Kondor*) which, it was claimed, produced a superior beverage without the use of a "binder," most coffee tablets containing some substance designed to keep the tablet intact.

Another prepared coffee, developed at the Mellon Institute in Pittsburgh in 1934 under the sponsorship of the Continental Can Co., is known as "flake coffee." Ground coffee is "flaked" by passing it through a roller mill under pressure by which means the particles are converted into thin, flat flakes averaging about 0.06 in. in diameter and about 0.003 in. in thickness. The sponsor claims that by means of this flaking operation the soluble constituents of the coffee become instantly accessible to the water, resulting in quick and efficient extraction.

Strictly speaking, there is no "substitute" for coffee but in all the big coffee-consuming countries, coffee additions and fillers have always been used. Large numbers of French, Italian, Dutch, and German consumers insist on having chicory with their coffee, just as do many Southerners in the United States. Roasted cereals are widely consumed in Europe as substitutes for coffee.

The chief commercial reason for using coffee additions and fillers is to keep down the cost of blends. For this purpose, chicory and many kinds of cooked cereals are most generally used, while roasted and ground peas, beans, and other vegetables are frequently employed in foreign countries. Before Parliament passed the Adulterant Act, some British coffee men used as fillers cacao husks, acorns, figs, and lupines, in addition to chicory and the other favorite fillers.

Up to the year 1907, when the United States Food and Drugs Act became effective, chicory and cereal additions were widely used by coffee packers and retailers in this country. With the enforcement of the law requiring that the label of a package so state when a filler is employed, the use of additions gradually fell off in most sections.

In botanical description and chemical composition, chicory, the most favored addition, has no relationship to coffee. When roasted and ground, it resembles coffee in appearance; but it has an entirely different flavor. However, many coffee drinkers prefer their beverage with this alien flavor added to it.

The most famous of Europe's coffee substitutes is made from roasted malt and unblushingly called *Malt Coffee.* In America the best known is straight cereal, formerly carrying the additional word "coffee" but not since 1906 when, with the advent of the Pure Food Law, all cereals masquerading as coffee were forced to drop the misleading word from their labels.

II. TEA

The word tea comes from the Chinese local Amoy dialect word *t'e,* pronounced "tay." In Cantonese it becomes *ch'a,* pronounced "chah." The name traveled in this form to Japan, India, Persia, and Russia. The Dutch brought the form *t'e* to Europe via Java. The English word "tea" was derived through the Dutch.

1. Early History

There is a Chinese legend which places the introduction of tea drinking in the reign of the mythological Emperor Shen Nung, about 2737 B.C., but the earliest credible mention is A.D. 350, when tea was described by Kuo P'o in the *Erh Ya* or ancient Chinese dictionary.

Mother Nature's original tea garden was in a portion of southeastern Asia which includes bordering provinces of southwestern China, northeastern India, Burma, Siam, and Indo-China.

Tea cultivation and the use of the beverage spread throughout China and Japan under the patronage of Buddhist priests, who sought a means of combating intemperance. Tea had its first handbook in the *Ch'a Ching*, written about A.D. 780. In it tea appears as a kind of cake made from leaves that had been steamed, crushed, and moulded. To prepare it, the cake was fired or toasted and shredded, after which it was steeped in salted boiling water. It is also recorded that tea was taxed under the T'ang dynasty in China A.D. 780.

The earliest notice of tea in Japanese literature dates from A.D. 593, and its cultivation from A.D. 805. Japan also had its book of tea, but not until A.D. 1200.

The first account of tea reached the Arabs A.D. 850; the Venetians in 1559; the English in 1598; the Portuguese in 1600. The Dutch brought the first tea to Europe about 1610; it reached Russia in 1618; Paris, 1648; and England and America about 1650.

2. Production

The world's production of tea totals around 2,000,000,000 lbs. annually. This is from an area of perhaps 4,000,000 acres. More than 4,000,000 laborers are engaged in its cultivation, plucking, and manufacture.

No one knows with accuracy how much tea China, the greatest producing country, contributes to swell the world total, and naturally this element of uncertainty is a handicap in determining the entire tea yield. Estimates generally vary from 500,000,000 to 900,000,000 lbs. per year. The more authoritative sources indicate a figure of about 600,000,000 lbs. a year at present. The world total was 1,657,309,000 lbs, in 1938.

While the total yield at best is an approximation, the amount actually placed on the world markets is obtainable from official reports of the various countries. According to these reports, the quantity marketed in 1939 was 875,703,000 lbs., the balance produced being retained for consumption in the producing countries, principally in China. These export figures are a better guide to the trend in world conditions, for they are available over many years.

The decrease in exports since 1932, when the total was 932,661,000 lbs., is directly attributable to the export restriction policy, established in 1933 as the result of an

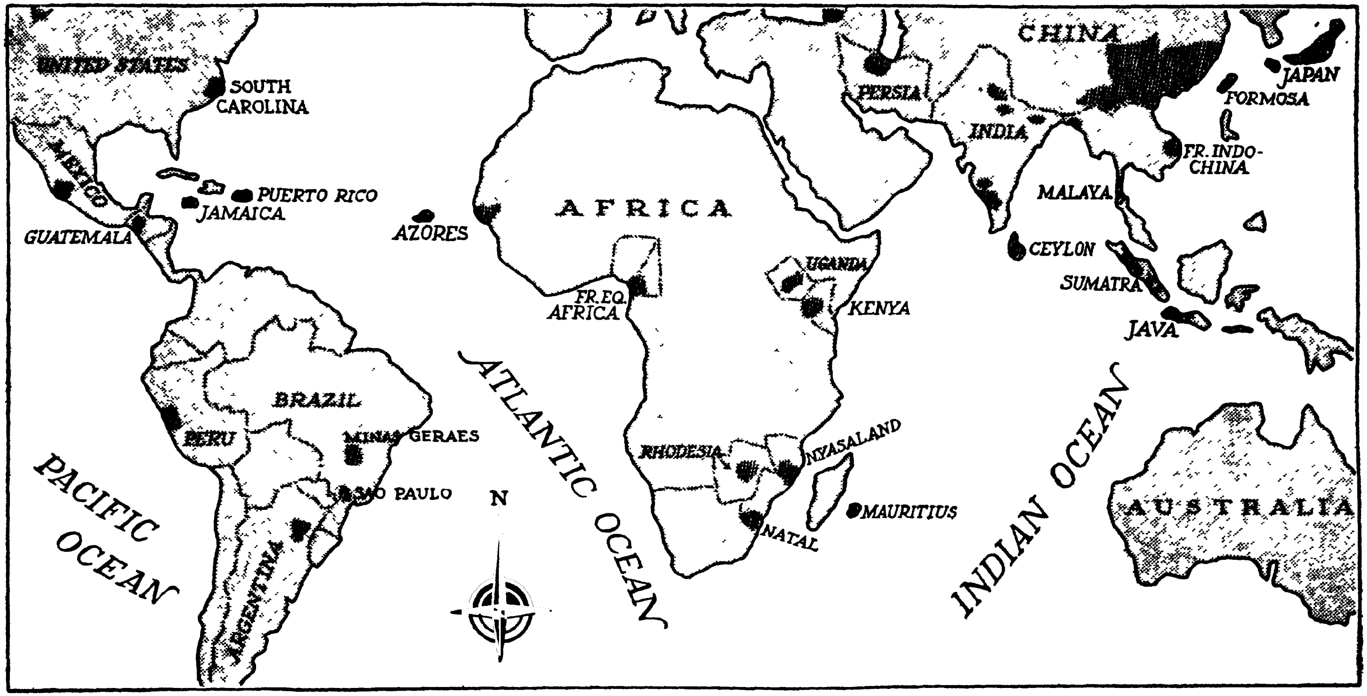


Fig. 79.—Tea map of the world showing distribution of the producing areas.

agreement entered into between India, Ceylon, and the Netherlands Indies to regulate tea exports for a period of 5 years. This agreement was renewed in 1938 for another 5 years.

The bulk of the world's tea is grown in Asiatic countries, and China is unquestionably the largest producer, being the source of almost one-half of the total, considered on an average of 5 years. India supplies about 23% of the total yield, Ceylon 13%, the Netherlands Indies 9%, Japan 5%, Formosa 1%, and all other areas less than 1%. See Fig. 79.

On an exporting basis, however, according to latest available figures (1939), India takes the lead with about 37%; Ceylon follows with 26%, the Netherlands Indies 18%, Japan with 6%, China 5 1/2%, Formosa 3%, and all other sources about 4 1/2%. Tea is grown in 23 countries.

3. Consumption

About half the world's tea crop finds its way every year to importing countries and is consumed there, barring the stocks held in their principal markets, the most important of which is London. The importing countries consuming tea in greatest bulk are the United Kingdom, the United States, Australia, and Canada, in the order named. In the early years of this century, however, Russia stood second in order.

The per capita consumption in the principal consuming countries (1939) follows: United Kingdom, 9.87 lbs.; New Zealand, 6.88 lbs.; Australia, 7.11 lbs.; Canada, 3.98 lbs.; Morocco, 3.03 lbs.; Netherlands, 3.35 lbs.; South Africa, 1.9 lbs.; Egypt, 1.09 lbs.; Iran, 0.77 lb.; United States, 0.74 lb.; Russia, 0.21 lb.; Germany, 0.17 lb.; France, 0.07 lb.

Tea consumption in the United States has been as high as 1.56 lbs. per capita (in 1897), but in recent years it has fallen off. In 1830 it was 0.53 lb. It reached its lowest point in 68 years in 1934 when it touched 0.59 lb. per capita. In 1940 it was 0.73 lb.

The United States imported 98,962,516 lbs. of tea in 1940. Netherlands Indies supplied 31.3%; Ceylon 26.2%; Japan 17.8%; British India 17.4%; China 5.9%; other countries 1.4%.

4. Botany

The tea plant was first classified by the Swedish botanist, Carl von Linné, 1707–1778, best known as Linnaeus, as *Thea sinensis*, in 1753. Since then the genus *Thea* has come to include the Linnaean genus *Camellia*, which is chiefly known for the familiar white or red flowers of the *Thea japonica*.

Botanists now consider Assam and China tea as equivalent tea-yielding plants, and so, with a bow to J. Sims, who first joined several species, the complete name of the tea plant is *Thea sinensis* (L.) Sims. Its botanical classifications are as follows: division—*Angiospermae;* class—*Dicotyledoneae;* order—*Parietales;* family—*Theaceae;* genus—*Thea;* species—*sinensis*.

The tea plant is an evergreen shrub, which, in its natural state, grows to a height of from 15 to 30 ft., but the tea planter keeps it pruned down to a height of from 3 to 5 ft. In its general appearance and the form of its leaf it resembles the myrtle; its blossoms are white with yellow anthers and suggest the wild rose. These are succeeded by the tea fruit containing 3 seeds.

The tea plant grows in the tropics from sea level up to 6000 ft.; in more temperate zones, where there is danger of frost, it must be kept to low elevations. As with coffee, however, it does best in the higher altitudes of the tropics. The commercial tea belt of the world lies within 42° north and 33° soutlh latitude.

Tea cultivation in India has been most successful along the line just north of the Tropic of Cancer in the latitude where the Chinese first achieved a notable success in China and Formosa. Originally tea seeds were brought from China to India, but later the indigenous variety was found in Assam. Intensive tea cultivation in Japan dates from the 12th century. The Dutch started its cultivation in Java in 1826. It became an important commercial factor in Formosa about 1867. Ceylon took it up after leaf disease had ruined its coffee industry in the '70's. Since then it has been cultivated on a small scale in the Caucasus, Natal, Nyasaland, Tanganyika, Rhodesia, Ethiopia, Kenya, Uganda, Portuguese East Africa, Iran, French Indo-China, Siam, Burma, Malaya, West Indies, Brazil, Colombia, Paraguay, Argentina, Peru, Chile, Mexico, Guatemala, Puerto Rico, the Azores, Australia, Mauritius, and even in South Carolina. But the main sources of world supply are India, Ceylon, China, Japan, Formosa, Java, and Sumatra.

5. Cultivation

Tea usually is grown from seed in nursery beds. The young plants are transplanted to the prepared tea fields when they are 6 to 8 in. high, usually in 6 months' time. They are set out in rows, 3 to 6 ft. apart. In 2 years they reach a height of from 4 to 6 ft. Then they are cut down to something less than a foot. By the end of the third year they are ready for plucking. Japanese and Formosan tea growers do not set out nurseries, but propagate their tea bushes by planting the seeds directly where they are to be grown or by layering, *i. e.*, transplanting rooted branches. Weeding, cultivation between the rows, and pruning go on regularly at different periods for 25 to 50 years, the average life of a tea bush. When in full bearing—about the tenth year—a bush yields as much as a quarter of a pound of leaf in its several flushes during the season.

The quality of the tea is at its best at different seasons in the various tea countries. Teas are plucked all the year around in southern India, Ceylon, Java, and Sumatra. Seasonal teas are those coming from northern India—the China greens, Congous, Japan teas, and Formosa Oolongs.

The plucking is done by native men and women who strip the leaves directly into bamboo baskets at their sides or who toss them deftly over their shoulders into baskets hung upon their backs by thongs passing over their foreheads. In northern India, during the first flush, only the tips of the new green shoots are plucked. There remain 3 or 4 leaves of which 2 may be plucked with the tip in the second flush and the remainder in the succeeding flushes. In Ceylon, a tea plucker, using both hands, plucks about 30,000 shoots a day. Approximately 3200 shoots are needed to make one pound of manufactured tea.

6. Classification of Teas

Generally speaking, teas may be divided into three classes: (*1*) fermented or black; (*2*) unfermented or green; (*3*) the semifermented or Oolong teas. Tea comes from practically the same plant in all countries, the differences in the various classes being due to methods of manufacture and local climatic, soil, and cultivation conditions. There are about 1500 different growths and over 2000 possible blends.

The fermented, or black, teas include the China Congou, or English Breakfast, teas, subdivided into North China Congou (black leaf) from Hankow and the South China Congou (red leaf) from Foochow; India, Ceylon, Java, African and, recently, Japan and Formosa blacks.

The unfermented, or green, teas include two main varieties—Chinas and Japans; also such green teas as may be manufactured in India, Ceylon, and Java.

The semifermented (Oolong) teas are obtained from Formosa and Foochow in China.

7. Manufacture

Black and green teas result from different manufacturing processes applied to the same kind of leaf.

After plucking, the leaf is withered by being spread on bamboo trays in the sun, or on withering tats within doors. Next it is rolled by hand or by machines. Then it is fermented in baskets or on cool cement floors under damp cloths. The firing process follows, in pans or baskets or in firing machines. The difference between black tea and green tea is the result of manipulation. Green tea is manufactured by steaming without fermentation in a perforated cylinder or boiler, thus retaining some of the green color. Black tea is allowed to ferment after being rolled and before firing. In the case of black tea the process of fermentation, or oxidation, reduces the astringency of the leaf and, it is claimed, develops the color and aroma of the liquor. In making green tea, the fermentation process is arrested by steaming the leaf while it is green and by rolling it lightly before drying.

Semifermented or Oolong teas have some of the characteristics of both black and green teas. They are allowed to wither in the process of manufacture and partially ferment.

(*a*) *Black Teas*

There are four principal operations in the preparation of black tea; namely, withering, rolling, fermenting, and firing. The effects of these operations may be summarized as follows:

Withering is a preliminary step to rolling, resulting in a desired loss of water, a flaccid condition of the leaf, and important changes in the constituents of the cell sap. Rolling imparts the characteristic twist, breaks the leaf cells, and exposes the juice to the air. During fermentation, the tea tannin is partly oxidized, and the leaf reddened. The essential oil is developed in fermentation. Firing stops fermentation.

Withering.—Withering methods vary somewhat in the principal black tea producing countries—British India, Ceylon, and Netherlands Indies. The atmosphere is practically always humid in Ceylon, Java, and Sumatra; whereas in British India it is only humid during the monsoon—a period of about 4 months. Accordingly, in Ceylon and Netherlands Indies, it is usual to wither the leaf in enclosed lofts, into which hot air, at not above 90° F., is passed at times when atmospheric conditions make a natural wither impossible. In some parts of British India—notably in Assam—withering lofts are generally unnecessary, as the leaf withers well in the open.

For withering, the leaves are spread thinly and evenly on racks called "tats." In some districts these are made of bamboo, which may or may not be covered with Hessian cloth, while in others they are of Hessian cloth alone, or of wire mesh. It requires approximately 1 sq. yd. of surface for withering each pound of fresh leaf, and the process takes from 18 to 24 hrs., depending on temperature and the humidity of the air. During withering, the leaves lose about one-third to one-half their weight by evaporation, become soft and flaccid, and emit an agreeable, characteristic, fruity odor.

At the same time a physical change takes place, but no apparent chemical change has yet been discovered. At present, the wither is judged by the moisture content, or loss of weight, of a certain quantity of leaf. In Assam, 100 lbs. of leaf are dried with an average temperature of 83° F. to about 65 lbs. In Ceylon, with a lower temperature the same weight is dried to about 55 lbs.

The withering must be slow and even; otherwise, the leaves, being thin, shrivel and blacken before the bud and stalk have hardly dried.

There is much discussion as to the best type of withering rack. Hessian or bamboo racks give an even wither, but require thin spreading, since most of the drying takes place from the upper surface of the rack. Wire racks take more leaf, because of the double drying surface, but have the disadvantage of allowing fine leaf to hang through and overdry. A drawback of Hessian racks is that they are likely to harbor harmful bacteria, and to get sour in prolonged spells of damp weather, especially in Assam where they are open to the air at the sides.

An even wither is essential to good tea making, and, to this end, the leaf must be thinly spread. In Ceylon, a common average of rack area per pound of green leaf is 10 to 12 sq. ft., although many factories have much greater space; some as much as 30 sq. ft. In India, the average is about the same as in Ceylon; but in the better tea areas, 25 to 30 sq. ft. per pound of leaf is common. In Java, the spreading is considerably thicker than in India or Ceylon.

Where weather conditions are unfavorable teas are withered artificially. Hot air from the drying room is conducted into the withering lofts and drawn over the leaf racks by means of fans.

In addition to loft control, many attempts have been made to dry the leaf artificially in machines constructed on the principle of tea-drying machines. The leaf is run through them on an endless tray system, and submitted to an air blast of about 110° F. It is thereby reddened, but the resulting made tea usually is "harsh" and inferior to naturally withered leaf.

As previously stated, the chemical changes which occur in the tea leaf during the withering process have not been completely elucidated and analyses fail to disclose any significant chemical differences between fresh and withered leaf; but the ease with which tannin, and other soluble bodies, may be extracted by boiling water increases as the wither proceeds. Thus, if fresh leaf is placed in cold water to which a drop of ferric chloride is added, no coloration takes place for several days. When withered leaf is so treated, however, a greenish coloration is obtained in a few minutes, showing immediate diffusion of the tannin from the leaf. The tannin content and soluble extract of the tea leaf are practically unaltered by withering. The *p*H of the cell sap also is unchanged, showing a value of 4.3–4.5 in all stages of the wither.

Many theories have been formulated as to the changes in the tannin compound when the leaf withers. The earlier theories on the subject considered the tannin in fresh leaf to be in the form of a glucoside, which, on withering, was hydrolyzed. The theory that tea tannin is in some way "protected" in the fresh leaf, and hydrolyzed on withering, is a convenient one, although experimental proof still is lacking.

If fresh leaf is infused, a greenish-yellow solution is obtained. As the leaf withers, the green color becomes less marked. The taste of these infusions has been reported on by tea tasters. According to these experts, fresh leaf infusion is "raw," while withered leaf infusion is "harsh." The exact significance of these terms is difficult to convey, and tasters, themselves, find great difficulty in explaining just what they mean. Rawness, however, appears to denote bitterness, and harshness apparently refers to a kind of astringency.

Rolling.—If the leaf is at all damaged or bruised, the infusions become red. These infusions are still pronounced raw by tasters, but "pungency" is also detected. Pungency normally appears as soon as the leaf is rolled, and it seems that this characteristic, which is akin to astringency, and "briskness"—another tea taster's expression—results from the oxidation, *i. e.*, fermentation, of the leaf. The "tannin red" compounds seem to be connected with pungency. This finds confirmation in the work of Peacock, who showed that the astringent principle of maté is due to the presence of phlobaphenes, or tannin reds.[6]

The object of rolling is to break the leaf cells and liberate the juices and enzymes sealed within. A secondary object is achieved when the tea is given its peculiar and well-known twist. As soon as the leaf cells are broken and the juices exposed to the air, fermentation begins. Oxygen is absorbed, a certain amount of heat is developed, the leaf turns from green to a copper-red color, and takes on an aroma peculiar to tea. These processes are spoken of, collectively, as fermentation. In the earlier days of tea manufacture, the leaf was rolled by hand after withering, as is done to this day with the Congous, or black teas, of China. For many years, however, machines have been used to do this work in India, Ceylon, and Netherlands Indies.

---

[6] J. C. and B. L. Peacock, *J. Am. Pharm. Assoc.*, **9**, No. 8 (1912).

A tea roller consists of a brass-covered table with battens attached to its surface, upon and against which an open-bottomed leaf box is given a circular, grinding motion, while an adjustable compression arrangement makes possible the application of any desired pressure of leaf against the surface and battens of the table.

Although the same mechanical tea rollers are used in India, Ceylon, and Java, the system of rolling is by no means the same in the three countries. In Ceylon and Java, the rollers revolve at about 45 revolutions a minute, and the roll may last, particularly in Ceylon, as long as 3 hrs. In India, the rollers make up to 70 revolutions a minute, and the normal rolling time is about 1 to $1^1/_2$ hrs.

The effect of slow and fast rolling has been studied, and, under favorable temperature conditions, no appreciable difference is noticeable in the liquors from teas made by the two methods. Slow rolling, however, involves long rolling, during which time the leaf, closed in, is likely to heat. In India, with the natural high temperatures, this heating must be avoided, and it is preferable during most of the season to get the roll finished and to spread the leaf thinly on the fermenting floor, rather than to keep it boxed up in a roller.

The slow roll in Ceylon is also connected with the full wither. It is observed that, the fuller the wither, the slower must be the roll to be effective.

So far as the appearance of the tea goes, slow rolling gives less broken tea with more "tips." Fast rolling apparently smears the tip with the juices from the other leaves much more than does slow rolling; the result being that much of the tip is indistinguishable from broken fine leaf.

In Ceylon, the leaf may be rolled as many as six times. After each roll the leaf is sifted, and fine leaf removed and taken to the fermenting room. In this manner the rolling process may take 3 hrs., and the leaf finally left in the roller may be less than half of the original.

In India, the roll consists of two parts. First, the light roll, lasting about half an hour, by means of which the shoot is broken up and the tip and fine leaf detached, so that, when the green leaf is sorted, the fine stuff may be separated and taken to the fermenting room. The second roll is hard, with the pressure cap of the roller down. This roll may last for an hour.

Experience has shown that hard rolling gives strength to the tea liquors, but may expose stalk. During rolling, heat is developed, but this is mainly the heat of fermentation and not the heat of friction. A large rise in temperature is avoided by raising the pressure cap of the roller every few minutes.

As the rolling proceeds, the leaf forms into balls, which, if fermented as such, give uneven colors, for the inside of the ball is shut off from the atmosphere, and remains greenish. These balls are broken when the leaf is green-sifted, after rolling. The green-sifters are known as ball-breakers and in Ceylon the process is regarded as one of aeration and oxidation. In India, the temperature usually is so much higher than in Ceylon, that the oxidation process goes fast enough without additional help, and green-sifting, apart from ball-breaking, is looked upon as a means of cooling the leaf after rolling.

The combined roll-breaker and green-leaf sifting machine, to which the leaf goes from the roller, has a roll-breaking hopper provided with beaters to break up the leafy lumps, and either a sifting tray or a cylindrical sieve of generous dimensions. Two sizes of sifting screen on one tray are used in many tea factories; screens having 3 and 4, or 4 and 5, or 4 and 6 holes per inch, respectively, being used in varying proportions to suit local conditions. Whether more of the coarse, or more of the fine screen is used, is open to individual determination; but there seems little doubt that the combination of two sizes is preferable to a tray fitted with but a single-sized screen.

Fermentation.—From the rollers and roll-breaker, the leaf, still more or less green and quite flaccid, goes to the fermenting room, where it is thinly spread on the cement, glass, or tile floor, or on glass or cement tables, to complete the process of oxidation begun and extensively developed during rolling. These fermenting spaces are washed down regularly with water, and anything in the nature of an ammoniacal smell is avoided. Although the process under discussion is given the distinctive title of "fermenting," in reality it marks the completion of that process.

During the fermentation of the tea leaf, oxidation brings about chemical changes which largely determine the flavor, strength, body, and color of its liquor; also the color of the infused leaves. The previously colorless tannin is transformed into red tannin and brown tannin, which give color to the liquor. The chemistry of tea fermentation is discussed by Roberts.[7]

Fermentation proceeds best between 70 and 80° F. (cold fermentation), although in Assam it is often difficult to keep the temperature down to 80° F. in the rains. In cold areas, such as Darjeeling and the Pengalengan plateau, the leaf is sometimes spread near the driers to aid fermentation. In Ceylon, it is often spread very thinly to insure a good air supply, and, in this manner, fermentation is hastened.

The humidity in the fermenting room is maintained at a degree close to the saturation point. This is sometimes brought about through the use of Hessian curtains hung roundabout the walls of the room from perforated pipes, which sprinkle water on the curtains; also by the use of wet cloths on frames placed a few inches above the fermenting leaf. Many factories have installed humidifiers similar to those used in cotton mills.

As a general rule, the shorter the fermentation, the more pungent the liquors; and the longer the fermentation, the softer the liquors, and the deeper their colors.

---

[7] E. A. Houghton Roberts, "Chemistry of Tea-Fermentation," in *Advances in Enzymology*, Vol. II. Interscience, New York, 1942, p. 113.

The chemical reason for this is that tannin in its natural state is colorless and pungent to the taste, but fermenting causes it to develop color and lose pungency.

The leaf is allowed to ferment until it arrives at the proper color, which is bright copper-red. The time, varying from $1^1/_2$ to $4^1/_2$ hrs., depends largely on the degree of the wither and the type of rolling. Locality also makes a difference; leaf from different gardens requires different types of fermentation. Only by careful experiment can the best time and character of ferment be determined.

Two main factors are concerned in the rate of fermentation of leaf, namely, temperature and air supply. The course of fermentation is largely influenced by the humidity of the atmosphere.

During fermentation many reactions are going on, and, although a high temperature may merely increase the rate of these reactions and lower the time necessary for fermentation, experience shows that, quite apart from taints arising from bacterial action or lost flavor, the best tea is made at low temperatures. It is possible that the chemical reactions making up fermentation proceed best at about 75° F., and that, at temperatures much removed from this, the different reactions are out of step for the production of the best tea.

The rolled leaf is spread in beds at a depth varying from $1^1/_2$ to 5 in. The thicker the spread, the less chance there is of air reaching the leaf, hence longer fermentation is required. However, the thicker the spread, the more the heat of fermentation is conserved, and the hotter the bed becomes. This second factor reduces the time necessary for fermentation, but the ultimate result of a thick spread is that a longer fermentation is necessary than with a thin one.

Considering that fermentation consists largely in the formation of tannin reds, which make for liquor and color of infusion, it follows that the longer the fermentation, the thicker will be the liquors at the expense of pungency, which is largely governed by the tannin, as opposed to the tannin red content.

Only rarely do teas show both marked pungency and strength of liquors. A tea buyer prefers one or the other of these qualities, and a tea possessing either definite pungency or strength has a better market value than one possessing both qualities, but not in a marked degree.

Firing.—When tea drinking was first adopted in Europe and America, and China tea formed the only crop of any consequence commercially, *kuo* firing in shallow iron pans and basket-firing in hourglass-shaped baskets over small charcoal fires were the methods followed. Hand methods still prevail in China, Japan, and Formosa, but with the greater consumption of tea from the newer tea-producing countries of India, Ceylon, and the Netherlands Indies there arose the necessity for firing in large quantities, and machine driers were installed.

There are various types of machine driers supplying artificial heat by different means. Some are fed automatically, the leaf falling on traveling trays which carry it from the top of the machine to the bottom, where it is discharged; others are provided with trays on which the leaf is spread and then placed in the machine, as bread is placed in a baker's oven.

The principle of drying tea consists in blowing or drawing a blast of hot, dry air up or down through an inclosed chamber through which the tea passes on trays. In the updraft machine the hot, dry air entering at the bottom of the chamber at a temperature of about 200° F. meets first the dry leaf, which is just leaving the machine. The air then passes through the trays bearing less dry tea. Finally, when the air leaves the machine, it is cooled to about 120° F. and is moist, having just passed the cold, wet, incoming leaf.

If tea is dried quickly in a dry atmosphere, it becomes case-hardened, and the center of the leaf remains moist. The result is that slow chemical changes take place, the tea loses quality, and sometimes even goes moldy.

Tea fermentation appears to be stopped at temperatures of about 150° F., or considerably lower in a blast of air. Thus, it has been found that if the incoming leaf meets a temperature of about 120° F. in the firing machine, the fermentation is effectively stopped. Sometimes, if the machine is overloaded, the air blast is cooled so that when it reaches the exit its temperature is below 120° F. In this case fermentation goes on for a time within the machine at an enhanced rate, and the leaf stews, producing a "flat," or overfermented, tea.

Leaf enters the firing machine with a moisture content of 50 to 65%, according to the degree of the wither. In India it is usual for the leaf to contain well over 60% of moisture at the time of firing, and the operation is carried out in two stages. First, the tea is fired until it contains about 30%, and then, with a second firing in another machine, the moisture is reduced to about 3%. It has been observed that firing to less than 30% moisture—a condition known as a 12-anna fire in India—is a dangerous practice, because it always involves "stewing" the leaf and the disadvantages which go with this condition.

In Ceylon and Java, the leaf usually is more fully physically withered than in India, and is completely fired in one operation.

From the chemical standpoint, the object of firing tea is to stop fermentation by destroying the enzymes and any microorganisms which may take part in the process. This destruction is brought about by heat and the removal of moisture. In addition to this action some of the substances present in the leaf are changed by heat to give the "malty" taste and smell of freshly fired tea. This change is known as "caramelization," and is similar to the change taking place when sugar is browned in a pan. Other substances, which have a cabbage flavor in fermented leaf, lose this undesirable quality when fired.

During the firing process, some valuable constituents of the tea are lost. This is, to a certain extent, unavoidable, but the loss may be minimized by careful adjustment of temperature and air blast of the firing machine. The chief loss is of those substances which give rise to flavor and aroma, and are volatile in steam. The higher the firing temperature, the greater the ease with which they evaporate along with the moisture, which is driven from the leaf. Teas fired below 170° F. are likely to "go off" on storing. In addition to a loss of flavoring substances, there is a loss in caffeine, but this is minimized by low firing.

Another factor, which sometimes is responsible for loss of aroma, is the "stewing" of tea in the drier. This follows from too thick spreading in the machine, or from a too weak air blast. It is probable that if steam is allowed to collect round the tea as it dries, there is a greater tendency for the essential oil, which is volatile in steam, to be lost, than if a full air blast is employed.

Attempts have been made to avoid these losses by drying tea in a blast of cold air, artificially dried by means of asbestos grids charged with calcium chloride. Although this process gives a very fine tea, it unfortunately gives one that will not keep unless it is partially dried by hot air. The double process of drying, both cold and hot, although making a good tea, is economically impracticable.

During firing, the proteins in the leaf are coagulated and rendered insoluble. These bodies, except in a degraded form, do not, therefore, enter into the tea infusion.

The red, fermented tea leaf turns black on firing, but when the leaf is infused, the red color is again observed.

At the conclusion of the firing process, the leaf is piled in heaps on the floor to cool, after which it is placed in bins or other receptacles, ready for sorting.

(*b*) *Green Teas*

Green teas are prepared in only three principal operations: namely, steaming or pan-firing, rolling, and firing. The difference between the manufacture of green tea and the manufacture of black tea is that green tea is not fermented. Leaf intended for manufacture into green tea is steamed, instead of being subjected to the slow process of natural withering, common to black tea preparation. Steaming or pan-firing, not only makes the leaf pliable, but sterilizes the leaf against fermentation and blackening.

Much of the tea made in China, and most of the Japan tea, is unfermented. A small quantity of green tea is made in northern India, and practically none in Java and Ceylon.

In China, green tea is manufactured by a process similar to that for black tea, except that the preliminary withering process is omitted and the leaf is immediately fired in pans at a temperature somewhat higher than in the case of black tea, and for a longer period. Fermentation thus having been made impossible, the rolling and firing follow.

The leaf destined to be made into green tea is generally plucked without stalk.

In Japan, it is usual to steam the leaf immediately after it is brought in and, the enzymes and microorganisms having thus been destroyed, to roll it until dry on paper trays heated by hot, metal plates. Although the rolling process is continuous, it is divided into four periods, during which moisture is steadily lost. Many factories employ electrically driven machines which duplicate, somewhat, the four processes used in hand rolling.

If rolling machines are employed, hot air is blown into the machine when it is boxlike or the plates are warmed if it is an open machine. Tea for export is refired in iron pans at a central station, and dried until it contains about 2% of moisture. The best tea is refired in baskets, as in China.

Much less information is available concerning the chemical changes which occur during the manufacture of green tea than is the case with black tea manufacture. The chief differences between black and green teas result from fermentation. In green tea most of the tannin is present in the original form, and the slight loss of tannin which occurs during manufacture is the result of incipient fermentation, or precipitation by proteins. Tannin red and tannin brown do not appear in a good green tea infusion, for the first step in manufacture seeks to avoid their formation.

The preliminary steaming, or heating, of the leaf kills any organisms which may give rise to the essential oil of tea, and, accordingly, green tea has no such aroma and flavor as is understood when speaking of black tea.

(c) *Oolong Tea*

Oolong tea has been described as a cross between green and black. The leaf is slightly withered before panning, and during this process a light ferment is allowed to develop. For this purpose, the leaf is spread 3 or 4 in. deep in large bamboo baskets, and placed in the shade, where it is frequently turned over for 5 to 6 hrs. The temperature of the leaf, during this time, ranges from 83 to 85° F. Finally, it changes in color and gives off a characteristic applelike odor, which is the signal for checking any further fermentation by drying.

Oolong tea is dried by panning for 10 min. in a *kuo,* or hot pan, set in the top of a brick or clay stove. The temperature of the pan is approximately 400° F. During this time the leaf is energetically stirred by hand or by a mechanical stirrer, to prevent scorching.

The leaf is rolled on a mat immediately after panning, the operation occupying about 10 min.

Oolong teas are fired in the type of baskets long familiar in tea manufacture. Formosa baskets are 30 in. high and 27 in. in diameter, open at both ends, and slightly drawn in at the waist where the tray for the tea rests. A circular hole in the ground, about 18 in. in diameter and 12 in. deep, contains the charcoal fire, from which all gas and flame have been burned before the tea is placed over it. The fire is covered with ashes, and the tea is dried for about 3 hrs., during which time the baskets are occasionally removed for the purpose of stirring the leaf.

This completes the country, or local, process of preparation. Subsequently, the leaf is collected by agents for the tea hongs, who send it to tea-firing godowns in the towns, where it is refired in baskets at a temperature of about 212° F. for from 5 to 12 hrs. The best-quality teas are given the longest firing.

When refiring has been completed, the tea is packed in lead-lined chests, with inner paper linings, pasted over with fancy paper, and wrapped in matting.

8. Trade Characteristics

The teas that grow and are plucked all the year round are those of southern India, Ceylon, Java, and Sumatra. The best qualities in southern India are the fermented teas produced in December and January. In Ceylon the finest-quality fermented teas are produced in the months of February, March, April, July, August, and September. The fermented teas of Java and Sumatra are at their best in July, August, and September.

Northern India's fermented teas are best when made from the second flushes of July, August, and September. In Assam and the Dooars, however, the finest teas are made in November and December. The first pickings from April to October of the North and South China blacks are the best. The best China greens are made in June and July although they are obtainable from June to December. In Japan the season is from May to October. There are several crops, but the first and second (May and June) are best. In Formosa there are five recognized crops: *viz.*, spring, summer (2), autumn, and winter. The season extends from April to November. The first and second summer crop teas (June and July) are the finest.

(*a*) *Fermented or Black Teas*

In a brief discussion of trade characteristics, it is to be observed that in Class I (fermented teas) the North China Congous (English Breakfast teas) are strong, full bodied, fragrant, and sweet liquoring, particularly those from the Moning, Ningchow, and Keemun districts. The dry leaves are hard, black, and aromatic. They are the Burgundies of China teas. The South China red leaf Congous are light in the cup, with small reddish and tippy leaf. The best-known districts are Pakling, Paklum, and Panyong. These are the clarets of China teas.

Fermented Indias, Ceylons, and Javas are generally graded according to leaf sizes as follows: Broken Orange Pekoe, Orange Pekoe, Broken Pekoe, Pekoe, Pekoe Souchong, Souchong, Fannings, and Dust.

India teas are named after certain districts; the best known being: Darjeeling, the finest and the most delicately flavored; Assam, a hard, flinty, well-made leaf with plenty of golden tip and a strong, pungent, colorful liquor; Travancore, resembling Ceylon tea; and Dooars, a tea much used for blending because of its soft, mellow liquor.

Ceylon teas are known chiefly by their garden marks. They also are divided into high grown and low grown. Generally speaking, the former are of fine flavor and aroma while the latter have a common, rough liquor and are lacking in flavor. The Ceylon dry leaf is soft, black, and tippy.

Java and Sumatra teas are known by the names of the gardens or estates producing them. In cup quality they grade very well, being soft and of medium strength. The leaf is black, well made, and tippy.

(*b*) *Unfermented or Green Teas*

In Class II (unfermented or green teas) those coming from Japan are prepared in several styles of leaf: Pan-Fired (straight), Guri (curled), Basket-Fired (longer spider-leg leaf), and Natural Leaf (formerly called "Porcelain-Fired"). Japans are graded in district types as well as for style and cup quality. The grades generally recognized in the trade are extra choicest, choicest, choice, finest, fine, good medium, medium, good common, common. There are also nibs, dust, and fannings. Japans are the white wines of teas.

China Greens are divided into Country Greens (Moyunes, Teenkais, Fychows, Soeyans, Wenchows, and Local Packs), Hoochows, and Pingsueys. All are prepared in the following styles or makes: Gunpowder (extra, first, second, third, fourth, fifth, sixth, seventh, and common), rolled round, "fine shotty" to medium; Pea Leaf (first and second), a bold, round rolled leaf; Imperial (first, second, third), large size round rolled; Young Hyson, long, rough, bold, and twisted leaf; and Hyson, small, well-made, curly leaf.

The China Country Greens have rich, clear, and fragrant cup qualities. Hoochows are rather light liquoring, flavory green teas of good appearance. Pingsueys are slightly metallic and usually poorer cup, but better style than Hoochows.

The India, Ceylon, and Java Greens produce a pungent light liquor and are usually made up into Young Hyson and Hyson styles.

(*c*) *Semifermented or Oolong Teas*

In Class III (the semifermented teas) the Formosa Oolongs are of fine flavor and very fragrant. They are known by district names and are graded as Standard, On Good, Good, Fully Good, Good Up, Good to Superior, On Superior, Superior, Fully Superior, Superior Up, Superior to Fine, On Fine, Fine, Fine Up, Fine to Finest, Finest, Finest to Choice, Choice. The trade also recognizes several intermediate gradings, such as Good Leaf, Fully Standard, Standard to Good, Strictly Superior, Choicest, and Fancy. The leaf is greenish brown and tippy. Sometimes Formosas are blended with Gunpowders or Japans to make a mixed (green and black) tea.

The Foochow Oolongs have thin medium flavor and toasty cup qualities. The leaf is long, rough, coarse appearing, and blackish.

For a full discussion of the trade values and cup characteristics of the principal kinds of teas buyers are referred to *All About Tea.*[8]

9. Chemical Aspects and Tea Brewing

The principal components of tea are caffeine, tannin, and essential oil. The first supplies the stimulating quality, which, because of its purity, is without harmful reaction; the second, the strength of body; and the third, the flavor and aroma, chief factors in determining cup value in a trade sense. The perfect cup of tea is one which contains, among other things, the maximum amount of stimulant, caffeine, with the minimum amount of tannin. This result is achieved by infusing the leaves in boiling water from 3 to 5 min.

Tea is something more than a beverage. It is an adjuvant food. It promotes appetite, and good digestion waits on appetite. Men and women drink tea because it adds to their sense of well-being. It not only smells good and tastes good, but it at once stimulates and refreshes.

---

[8] W. H. Ukers, *All About Tea*. Vol. I, Tea and Coffee Trade Journal Co., New York, 1935, p. 223.

In China and Japan, the brew is usually so weak that the beverage becomes little more than a thirst quencher. In the British Isles, Australasia, North America, and Holland, where most of the black tea of India, Ceylon, and Java is consumed, the beverage is drunk primarily for its stimulating effect and then for the peculiar sour-harsh taste or pungency given by the astringent tannin and tannin compounds which are present in a black tea infusion. This taste becomes palatable by use. The alkaloid caffeine, responsible for the stimulus in tea, has a slightly bitter taste when taken in medicinal quantities, but the small amount present in the usual cup of tea is practically tasteless.

TABLE 213

CAFFEINE, TANNIN, AND SOLUBLE SOLIDS EXTRACTABLE BY A 5-MIN. INFUSION OF MANY SAMPLES OF TEAS FROM JAVA, JAPAN, CHINA, AND INDIA[a]

Infusion of	Caffeine, %	Tannin, %	Soluble solids, %
Java black teas	2.7–4.4	6–20	16–26
Japan green teas	2.0–3.3	4–12	16–26
China black teas	2.0–3.7	5–10	16–22
Formosa Oolong teas	3.1–3.7	12–23	23–25
India black teas	2.0–3.0	6–10	22–25

[a] J. J. B. Deuss, *L'Agronomie Coloniale*, Paris (August, 1924), No. 80 and C. R. Harler, *The Culture and Marketing of Tea*. Oxford University Press, New York, 1933.

Caffeine taken in small doses increases mental and muscular power and has no depressent aftereffect on the system. Tannin in large doses has a deleterious effect on the mucous membrane of the mouth and on the alimentary canal, but in the amount present in a cup of carefully made tea its harmful effect is negligible.

The ideal preparation of tea, then, is one which extracts a maximum of caffeine and a not excessive amount of tannin. Such a preparation also conserves the aroma and flavor—evanescent qualities easily lost by careless preparation.

From the chemist's point of view the two main essentials in tea brewing are fresh water, freshly boiled, and a 3- to 5-min. infusion. The tea used should be suitable to the water of the locality in which one lives. Tea infuses more readily in soft water than in hard. Small, thick, full teas are required for soft waters; brisk, full, flavory teas where the water is hard. Liverpool water, which comes from the Welsh mountains nearby, is said to be the softest water in England and the best for making tea.

Water fresh from the tap is the first essential. The second is to bring it to a bubbling boil and pour it at once upon the tea leaves. To delay means that the water will go flat, and flat water will spoil the finest tea. Hence, the discouraging concoctions on steamships and railways.

Generally speaking, a 5-min. infusion is best for fully fermented teas like those of India, Ceylon, or Java, although Mr. George F. Mitchell, former U. S. Supervising Tea Examiner, reached a slightly different conclusion in a series of tests some years ago at the United States Department of Agriculture.

These investigations demonstrated that a perfect cup of tea from a chemical standpoint was, unfortunately, not a perfect cup of tea from the consumer's standpoint. From a chemical standpoint the length of time in which the maximum amount of caffeine and total soluble matter was extracted, with the minimum amount of tannin, averaged 3 min. after boiling water had been poured on the leaves. After that time more tannin was extracted, with only a small amount of caffeine. Usually, however, this makes a very "skinny" cup of tea, lacking body and a certain amount of pungency that is desired by all tea drinkers; and, of course, if cream or milk is added to this beverage it takes away from the small amount of pungency, so that the cup appears even less desirable. For this reason Mr. Mitchell concluded it best to brew tea that is to be drunk without cream or milk from 3 to 4 min., and that to be drunk with cream or milk from 4 to 5 and even 6 min., as in some teas the real flavor does not come out until after a 6-min. infusion.

Messmer, of Frankfort-am-Main, evolved a method for making tea which, while scientific, seems "too bright and good for human nature's daily food." Messmer's method is to put one teaspoonful of leaf into a previously warmed china or earthenware pot, then pour upon it sufficient actively boiling water to cover it, and permit all the leaves to expand in infusion. After 5 min. the extract is poured off into a smaller pot. The procedure is then repeated, but the infusion lasts for only 3 min., after which the second infusion is added to the first brew. In the cup more boiling water is added according to the strength desired. By this method one obtains the maximum of stimulant, flavor, and aroma and the minimum of tannin, in happy combination.

Milk should be added to tea. Not only does this mellow the taste and add body to the liquor, but the casein in the milk renders the tannin insoluble.

The addition of sugar is a matter of taste. Many people do without it because it tends to mask the characteristic taste of tea.

10. Tea "Substitutes"

As in the case of coffee, there can be no substitute for tea. Besides the leaves of the tea shrub, the flowers are sometimes dried and a beverage made from them in the same way in which the leaves are brewed. Tea flowers may be said to represent a surrogate for tea.

(a) *Maté*

Perhaps the most important surrogate is *maté*, also known as *yerba maté*, Paraguay tea, or Brazilian tea. It is made from the dried leaves of *Ilex paraguayensis*, a shrub belonging to the family of *Aquifoliaceae*. The harvesting of maté is done principally by Indians, who climb the trees and cut off the leafy branches with knives. The branches are bundled together, and pulled through an open fire, so that the leaves are withered, but not scorched. After this treatment, the faggots are taken to the factory for the final drying, which takes from 14 to 16 hrs. The next step is to pound the leaves into a coarse powder, after which the powder is packed into bags for market. Occasionally, the drying is done in iron pans over brick ovens, in much the same way that tea is dried in China.

Maté is made in a small calabash about the size of a large orange, the top being open. Sugar and a little hot water are first placed in the gourd, the maté is added, and finally the vessel is filled to the brim with boiling water or heated milk. A little burnt sugar or lemon juice sometimes is added in place of milk.

The beverage is drunk by means of a small tube, 6 to 7 in. long, formed either of metal or of reed. It has at one end a bulb of extremely fine basket-work or of metal, perforated with minute holes. The maté is sucked up through the tube, which is called a *bombilla*. In family circles, the gourd and the *bombilla* are passed from hand to hand. Maté also may be brewed as tea and drunk from cups.

(*b*) *Cassina*

A beverage called *cassina* is made from the cured leaves of a caffeine-containing species of holly, *Ilex cassine*, indigenous to North America. The Bureau of Chemistry of the United States Department of Agriculture has developed a process of curing the leaves. The product is made into green cassina, black cassina, and cassina maté, the last resembling very closely yerba maté. Both maté and cassina contain anywhere from 1 to 1.60% caffeine and about 8% tannin.

The list of tea substitutes is well-nigh endless. Most countries have their peculiar "teas," and the exigencies of the first and second World Wars caused numerous ancient formulas to be raked up and new ones added.

(*c*) *Adulterations*

The most common adulteration consists of the addition of leaves of other plants. Leaves which, by their nature, have an astringent taste and a toothed border are most commonly used. Some of these are the leaves of the beech (*Fagus sylvatica*), hawthorn (*Crataegus oxyacantha*), *Camellia sassangua*, sloe (*Prunus spinosa*), and the *Chloranthus inconspicuus*. However, trusting to the indifference of the consumer, leaves of the oak, poplar, maple, and other trees, which bear not the slightest resemblance to tea, also have been employed.

Another formerly widespread adulteration was the addition of extracted tea leaves. The leaves were collected from restaurants, hotels, etc., and dried. Then they were mixed with real tea. Sometimes no fresh tea was added, but the spent leaves were impregnated with catechu, caramel, campeachy wood, indigo, Berlinblue, curcuma, humus, graphite, etc. The so-called *Thé de caravane*, *Spar-tea*, and *Königs-tea* are said to have been made in this way. A so-called *Caucasian tea* is a mixture of extracted tea leaves and leaves of the *Vaccinium arctostaphylos*. This sort of adulteration defies microscopic detection, but often can be discovered by chemical means; particularly determinations of hot water extract, tannin, and total and water-soluble ash.

Numerous adulterants have been used to "load" tea to increase its weight. Among these are clay, gypsum, iron filings, sand, etc. Chemical analysis will quickly reveal these.

Selected Bibliography

Fox, J. J., "Caffeine, Tea and Coffee" in *Allen's Commercial Organic Analysis.* Vol. VII, Blakiston, Philadelphia, 1930.

Ukers, W. H., *Coffee Merchandising.* 2nd ed., Tea and Coffee Trade Journal Co., New York, 1930.

Ukers, W. H., *All About Coffee.* 2nd ed., Tea and Coffee Trade Journal Co., New York, 1935.

Ukers, W. H., *All About Tea.* Tea and Coffee Trade Journal Co., New York, 1935.

Wiley, H. W., *Beverages and Their Adulteration.* Blakiston, Philadelphia, 1919.

Chapter XXIII

FLAVORS, SPICES, AND CONDIMENTS

LEE WORRELL
Ann Arbor, Michigan

The skillful use of suitable flavoring materials constitutes a major part of the art of successful food preparation. A diet composed of isolated fats, carbohydrates, and proteins, on which we depend almost entirely for energy, growth, and tissue repair, would prove to be quite insipid. Although flavoring materials are naturally present in many common foodstuffs, palatability is usually enhanced by the addition of various substances, having in themselves little or no nutrient value, but which add zest and variety to the diet and materially aid digestion by stimulating the flow of the digestive secretions. These substances, generally termed food flavors or condiments, include a large number of materials, many of which are totally unrelated chemically, but which are grouped together because of a similarity of usage. Among them are the spices, essential oils and flavoring extracts, vinegars, and mayonnaise and other salad dressings.

I. SPICES

The Federal Food and Drug Administration[1] has defined spices as "aromatic vegetable substances used for the seasoning of food. They are true to name, and from them no portion of any volatile oil or other flavoring principle has been removed."

Usually the spice consists of the leaf, flower, bud, fruit, seed, bark, rhizome, or other plant part which has been dried and is used either in the entire form or after grinding to a powder. In these forms spices were probably used much earlier than any of the other condiments. They have occupied an important place in world commerce for many centuries. In fact, the exploration of many parts of the world was due to attempts to find easier and shorter routes to the Orient for the purpose of obtaining spices. It has been pointed out repeatedly that America was accidentally discovered by Columbus in his search for an all-water route to India and the East Indies. During the period of the early explorations the demand for spices was great in western Europe, when strong flavoring agents were needed to disguise the odors and tastes accompanying the partial decomposition of foodstuffs which often occurred.

---

[1] U. S. Dept. Agr., Food and Drug Admin., Service and Regulatory Announcements, Food and Drug No. 2 (1936).

Until the start of the present war with Japan, the United States had always been a large importer of spices from the Orient. In 1940 (the last year for which complete statistics are available) we imported over 80,000,000 lbs. of spices, the declared value of which exceeded $13,000,000.[2] A large share of our annual consumption is accounted for by the meat-packing industry, but some spices, particularly pepper and mustard, are still purchased in large amounts by the ultimate consumer. Almost 20% of our total importation of spices is composed of black pepper.

1. Chemical Composition

Since the spices are composed of various parts of a large number of different plants, little can be said in a general way about their composition. Most spices owe their flavoring properties to their content of volatile oil; but this is not true of all, as will be shown when they are discussed individually. In some cases a fixed oil is chiefly

TABLE 214

ANALYSES OF PURE SPICES[a]

Spice	Moisture, %	Total ash, %	Water-sol. ash, %	Ash insol. in HCl, %	Vol. ether ext., %	Nonvol. ether ext., %	Alcohol ext., %
Black pepper	Max. 12.95	6.52	3.20	1.19	1.60	10.37	11.86
	Min. 10.63	3.09	1.75	0.00	0.65	6.86	8.47
	Av. 11.96	4.76	2.54	0.47	1.14	8.42	9.62
White pepper	Max. 14.47	2.96	0.80	0.20	0.95	7.94	8.55
	Min. 12.72	1.03	0.28	0.00	0.49	6.26	7.19
	Av. 13.47	1.77	0.47	0.10	0.73	6.91	7.66
Cayenne pepper	Max. 7.08	5.96	4.93	0.23	2.57	21.81	27.61
	Min. 3.67	5.08	3.30	0.05	0.73	17.17	21.52
	Av. 5.73	5.43	3.98	0.15	1.35	20.15	24.35
Ginger	Max. 11.72	9.35	4.09	2.29	3.09	5.42	6.58
	Min. 8.71	3.61	1.73	0.02	0.96	2.82	3.63
	Av. 10.44	5.27	2.71	0.44	1.97	4.10	5.18
Cinnamon	Max. 10.48	5.99	2.71	0.58	1.62	1.68	13.60
	Min. 7.79	4.16	1.40	0.02	0.72	1.35	9.97
	Av. 8.63	4.82	1.87	0.13	1.39	1.44	12.21
Cassia	Max. 11.91	6.20	2.52	2.42	5.15	4.13	16.74
	Min. 6.53	3.01	0.71	0.02	0.93	1.32	4.57
	Av. 9.24	4.73	1.68	0.56	2.61	2.12	8.29
Cloves	Max. 8.26	6.22	3.75	0.13	20.53	6.67	15.58
	Min. 7.03	5.28	3.25	0.00	17.82	6.24	13.99
	Av. 7.81	5.92	3.58	0.06	19.18	6.49	14.87
Allspice (pimento)	Max. 10.14	4.76	2.69	0.06	5.21	7.72	14.27
	Min. 9.45	4.15	2.29	0.00	3.38	4.35	7.39
	Av. 9.78	4.47	2.47	0.03	4.05	5.84	11.79
Nutmeg	Max. 10.83	3.26	1.46	0.01	6.94	36.94	17.38
	Min. 5.79	2.13	0.82	0.00	2.56	28.73	10.42

[a] A. L. Winton, A. W. Ogden, and W. L. Mitchell, Conn. Agr. Expt. Sta., *Ann. Rept.* (1898). M. B. Jacobs, *Chemical Analysis of Foods and Food Products.* Van Nostrand, New York, 1938, p. 334.

---

[2] U. S. Dept. Commerce, Bur. of Foreign and Domestic Commerce, Monthly Summary of Foreign Commerce of the U. S. (Dec., 1940).

responsible for the pungency, and in many cases no one single component can be singled out as responsible for it; rather the flavor of the spice seems to be due to the intimate natural blending of the flavors of a great number of different components, alcohols, esters, phenols, and their derivatives, terpenes, organic acids, sulfur-containing compounds, alkaloids, resins, and some which have yet to be identified. Since the content of these flavoring principles varies with the locality in which the spice is grown and also from year to year within the same locality, a great many different grades of any one spice may occur and standards must necessarily be broad.

TABLE 214

ANALYSES OF PURE SPICES (*Continued*)

Spice	Reducing matter,[b] %	Starch by diastase, %	Crude fiber, %	Protein, %	Total N, %	Cold water ext., %	Lime, %
Black pepper	Max. 43.47	39.66	18.25	13.81[c]	2.53	...	...
	Min. 28.15	22.05	10.75	10.50	2.03	...	...
	Av. 38.63	34.15	13.06	12.05	2.26	...	. .
White pepper	Max. 64.92	63.60	4.25	11.19[c]	2.13	...	...
	Min. 56.43	53.11	0.54	10.44	1.95	...	...
	Av. 59.17	56.47	3.14	10.89	2.04	...	...
Cayenne pepper	Max. 9.31	1.46	24.91	14.63	2.34	...	. .
	Min. 7.15	0.80	20.35	13.31	2.13	...	.
	Av. 8.47	1.01	22.35	13.67	2.18	...	...
Ginger	Max. 62.42	60.31	5.50	9.75	1.55	17.55	3.53
	Min. 53.43	49.05	2.37	4.81	0.77	10.92	0.20
	Av. 57.45	54.53	3.91	7.74	1.23	13.42	0.80
Cinnamon	Max. 22.00	...	38.48	4.06	0.65		
	Min. 16.65	...	34.38	3.25	0.52		
	Av. 19.30	...	36.20	3.70	0.59		
Cassia	Max. 32.04	...	28.80	5.44	0.87		
	Min. 16.65	. .	17.03	3.31	0.53		
	Av. 23.32	. .	22.96	4.34	0.69		
						Oxygen absorbed by aqueous extract	Quercitannic acid equivalent to oxygen absorbed
Cloves	Max. 9.63	3.15	9.02	7.06	1.13	2.63	20.54
	Min. 8.19	2.08	7.06	5.88	0.94	2.08	16.25
	Av. 8.99	2.74	8.10	6.18	0.99	2.33	18.19
Allspice (pimento)	Max. 20.65	3.76	23.98	6.37	1.02	1.59	12.48
	Min. 16.56	1.82	20.46	5.19	0.83	1.03	8.06
	Av. 18.03	3.04	22.39	5.75	0.92	1.24	9.71
Nutmeg	Max. 25.60	24.20	3.72	7.00	1.12	...	.
	Min. 17.19	14.62	2.38	6.56	1.05	...	...

[b] By direct inversion, calculated as starch.

[c] Total nitrogen less nitrogen in nonvolatile ether extract × 6.25.

In addition to their individual flavoring components, all spices contain at least some of the usual components common to most plant products, such as protein, crude fiber, ash, starch, tannin, etc. These are usually determined by the general methods used for the proximate analysis of plant products. Volatile oil can be determined by steam distillation and subsequent actual measurement of volume or it can be reported as the volatile ether extract. The nonvolatile ether extract is composed chiefly of fixed oil and in some cases small amounts of resins. The moisture determination is complicated by the volatile oil content and is usually reported as loss at 110° C. less the volatile ether extract. The detailed procedures for these as well as other special determinations are given in *Methods of Analysis* of the A. O. A. C.[3]

2. Adulteration

Spices, because of their nature, have always been subject to considerable adulteration and at the present time when our sources of supply for many spices have been temporarily disrupted, an even greater amount of adulteration may be expected. Several means have been used. The substitution of more common or less expensive spices for the rarer or more costly ones is relatively common. Gross adulteration by the addition of a great variety of different materials such as ground hulls from various fruits, sawdust, ground fruit seeds and nut shells, and other readily available waste materials is still practiced. Diluents such as starch, ground roasted cereal grains and partially or totally exhausted marcs may be added. Through careless methods of collection and cleaning, dirt, sand, twigs, stems, outer bark, and other extraneous materials may be present in excessive quantities.

By a combination of chemical and microscopical examinations it is usually quite easy to detect any of the above-mentioned adulterations.

The A. O. A. C. *Methods of Analysis*[3] (1940) gives a tentative method for a general microscopic examination of spices. The examiner needs at least a general knowledge of plant histology and must acquire a rather complete knowledge of the microscopic appearance of the various spices and of their common adulterants. In such books as *Microscopy of Vegetable Foods,*[4] *Structure and Composition of Foods,*[5] *Textbook of Pharmacognosy,*[6] and *Microanalysis of Powdered Vegetable Drugs*[7] are found detailed drawings of the characteristic structural elements of the individual spices and of some of the more common substances used for adulteration. However, skill and confidence in the microscopic examination are best gained by a systematic microscopic study of a large number of authentic samples of the various spices under consideration and of the materials used for their adulteration. With some experience one can easily detect substitution or adulteration by the addition of any of the usual waste materials by differences in the histologic structure. Excessive amounts of sand or dirt can frequently be shown by this method also. The microscopic examination fails to detect adulteration by the use of spice from which the flavoring principles have been removed by extraction. Obviously no characteristic differences in structure could be found in such cases; however, a very low total ether extract would be found during the course of the chemical examination. In most types of adulteration high ash and crude fiber values are found, along with low ether extracts. Leach,[8] Jacobs,[9] Winton,[10] and Woodman[11] all give a number of tables showing the results of chemical analyses of authentic samples of various spices.

---

[3] *Methods of Analysis.* Assoc. Official Agr. Chemists, Washington, D. C., 1940.

[4] A. L. Winton and K. G. B. Winton, *Microscopy of Vegetable Foods.* Wiley, New York, 1916.

[5] A. L. Winton and K. G. B. Winton, *Structure and Composition of Foods.* Vol. IV, Wiley, New York, 1939.

[6] H. W. Youngken, *Textbook of Pharmacognosy.* Blakiston, Philadelphia, 1943.

[7] A. Schneider, *Microanalysis of Powdered Vegetable Drugs.* Blakiston, Philadelphia, 1921.

3. Allspice (Pimento)

Allspice consists of the dried, nearly ripe fruit of *Pimenta officinalis* Lindley. This plant is a beautiful evergreen tree, varying from 20 to 40 ft. in height and found growing in the West Indies and along the coastal regions of southern Mexico, Central America, and northern South America. It is native to the island of Jamaica, being the predominating tree there; most of the commercial supplies are exported from Kingston.

The fruit is a round berry about the size of a small pea. It is collected in the late summer when fully grown but not yet ripe. The natives break off the small twigs and stems bearing the fruit and dry them in ovens or kilns. Formerly the drying was done on racks in the sun, but heavy dews and rains caused many losses. During the drying process the berries change from green to dark brown in color, lose about one-third of their weight, and become quite hard.

The flavoring component is a volatile oil, present in amounts of 3 to 4%, which contains eugenol and closely related phenols and which has an aroma similar to a mixture of cloves, cinnamon, and nutmeg. The Food and Drug Administration standards[12] require that allspice contain "not less than 8% of quercitannic acid (calculated from the total oxygen absorbed by the aqueous extract), not more than 25% of crude fiber, not more than 6% of total ash, nor more than 0.4% of ash insoluble in hydrochloric acid."

4. Anise (Aniseed) and Star Aniseed

These fruits are not at all closely related botanically yet yield volatile oils which as far as aroma and flavoring properties are concerned are indistinguishable.

Anise is the dried ripe fruit of *Pimpinella anisum* L., an annual herb of the parsley family which usually grows to the height of about 1 ft. and bears long inflorescences of small white flowers. The habitat of the plant is probably Asia Minor but it is extensively cultivated in most of the countries of Europe and to some extent throughout all temperate regions of the earth. Commercial supplies have been obtained until recently chiefly from the countries of southern Europe, Spain, France, Italy, and Russia.

The plants are grown from the seed and are cultivated in rows. Collection occurs when the fruits first begin to ripen; the stalks are mowed or pulled and stacked in the sun until ripening is complete. After being threshed the fruits should be carefully cleaned. They are small, ovoid, slightly elongated, hairy, ridged cremocarps, each usually bearing a small portion of its stem.

---

[8] A. E. Leach, *Food Inspection and Analysis*. Revised by Winton. Wiley, New York, 1920.

[9] M. B. Jacobs, *Chemical Analysis of Foods and Food Products*. Van Nostrand, New York, 1938.

[10] A. L. Winton and K. G. B. Winton, *Structure and Composition of Foods*. Vol. IV, Wiley, New York, 1939.

[11] A. G. Woodman, *Food Analysis*. McGraw-Hill, New York, 1941.

[12] U. S. Dept. Agr., Food and Drug Admin., Service and Regulatory Announcements, Food and Drug No. 2 (1936)

Star anise consists of the dried ripe fruit of *Illicium verum* Hook., an evergreen tree of the Magnolia family, growing to a height of about 20 ft., which is grown under cultivation in southern China, Indo-China, Japan, the Philippines, and to some extent in the West Indies. In recent years most of the importations of the United States have been from China.

The fruits, which are picked when mature and either dried or immediately distilled for their volatile oil content, are flattened rosettes composed of 6 to 8 (infrequently more) woody, brown carpels. These are usually split along the upper edge, exposing a shiny, brown seed.

The flavoring principle of both anise and star anise is a volatile oil the chief component of which is anethole, although small amounts of numerous other compounds have also been reported. The Food and Drug Administration standards require that anise contain "not more than 9% of total ash, nor more than 1.5% of ash insoluble in hydrochloric acid" and that star anise contain "not more than 5% of total ash."

5. Caraway (Caraway Seed)

Caraway is the dried, ripe fruit of *Carum carvi* L., a biennial herb of the parsley family. It has been cultivated for many years in practically all of Europe, except the Mediterranean region, and to some extent in northern portions of the United States. Dutch ports have been the point of exportation for most of the commercial supply.

During the second season of growth, when the fruits have begun to ripen, the plants are mowed and dried; then the fruit is separated by threshing, carefully cleaned, and packed in bags. The fruit, or "seed" as it is commonly called, is a cremocarp consisting of two long, narrow carpels which are dark brown, compressed at the sides, and occasionally curved. They usually become separated during threshing or storage.

Caraway contains as its chief flavoring principle usually about 5% of a volatile oil. This is composed almost entirely of *d*-carvone, which is present to the extent of 50 to 60%, and *d*-limonene; small amounts of other related compounds are also usually present. The standards of the Food and Drug Administration require that caraway contain "not more than 8% of total ash, nor more than 1.5% of ash insoluble in hydrochloric acid."

The fruits are frequently used in bread, rolls, and cookies and the volatile oil provides the characteristic flavor of kümmel.

6. Cardamom (Cardamon)

True cardamom consists of the dried, nearly ripe fruit of *Elettaria cardamomum* Maton; and cardamom seed consists of the dried seed of this species. The United

States Pharmacopoeia XII further requires that the seed be recently removed from the capsules. The fruits of a number of species of *Amomum* have in the past been marketed under the name "Cardamom" but have markedly inferior flavoring properties. *Elettaria cardamomum* is a large perennial herb of the ginger family which is cultivated extensively on the island of Ceylon and along the Malabar coast of India. Recently small plantings have been made in Central America. A number of commercial varieties of true cardamom, based both upon the size of the capsule and the locality in which it is grown, are recognized. The best grades are usually obtained from Ceylon.

The fruit is collected when nearly ripe, graded as to size, bleached either by sunlight or by chemical agents such as sulfur dioxide, and then dried and cured in the sun. It consists of an almost white, oval, papery capsule from 1 to 3 cm. in length, bearing a number of small, irregularly shaped brown seeds.

The flavoring component is a volatile oil present to the extent of about 4%, containing cineol and terpineol. The standards of the Food and Drug Administration require that cardamom seed contain "not more than 8% of total ash, nor more than 3% of ash insoluble in hydrochloric acid."

7. Celery Seed

Celery "seed" consists of the dried ripe fruit of *Apium graveolens* L., a biennial herb of the parsley family which probably is indigenous to southern Europe but which is cultivated in many countries of the world for its leaves and stalks. In its second year of growth the plant bears clusters of small, white flowers which are lost as the fruit ripens. Most of the commercial output of celery seed was supplied by southern France before the war.

The fruit is collected when fully ripe and dried. It is a very small, brown, almost spherical cremocarp, the carpels usually being found separated when the spice is purchased. The leaves are also sometimes dried, powdered, and used in mixed spices.

Celery seed contains about 15 to 20% of a fixed oil, and only about 2 to 3% of a volatile oil; but the flavoring properties are due almost entirely to components of the latter. Although *d*-limonene seems to be the chief component, the characteristic flavor is thought to be due to small amounts of a number of alcohols, acid anhydrides, and lactones. The standards of the Food and Drug Administration require that celery seed contain "not more than 10% of total ash, nor more than 2% of ash insoluble in hydrochloric acid."

8. Cinnamon

The barks of different species of the genus *Cinnamomum* are used as spices and there does not appear to be complete agreement with respect to the nomenclature.

Ceylon cinnamon or true cinnamon is the dried inner bark of cultivated varieties of *Cinnamomum zeylanicum* Nees.

Saigon cinnamon or cassia, according to the standards of the Food and Drug

Administration, is the dried bark of cultivated varieties of *Cinnamomum cassia* (L.) Blume; but the U. S. P. XII and *Standardized Plant Names*[18] agree that Saigon cinnamon is the dried bark of *Cinnamomum loureirii* Nees while cassia or Chinese cinnamon is the dried bark of *Cinnamomum cassia* (Nees) Nees ex Blume.

Batavia cassia or Malay cinnamon, the dried bark of *Cinnamomum burmani*, is not recognized as a spice at the present time although it may occasionally occur as an adulterant.

Cinnamomum zeylanicum is native to the island of Ceylon and when found growing wild it is a large evergreen tree, the young leaves of which are first scarlet and then change to dark green. All of the true cinnamon on the market now, however, is obtained from cultivated plants. The trees are cut back at frequent intervals to form stumps and from these a number of rapidly growing shoots arise. When the shoots are about 2 years old they are cut off and their bark removed by making two longitudinal incisions and peeling the young bark from the shoot. The bark is allowed to cure in piles for a few days during which it softens. The outer gray bark is then removed by scraping; and several of the thin sheets of inner bark are stacked and dried. During the drying process the bark shrinks and curls, and the final result is a firm cylinder or "quill." These are cut to convenient lengths, the ends being trimmed and shaped, and are packed into bundles for exportation.

The characteristic flavor of Ceylon cinnamon, which is quite different from that of the other cinnamons, is due to about 1% of volatile oil which, according to the Food and Drug Administration standards, should contain "not less than 65% by weight of cinnamic aldehyde and not more than 10% by weight of eugenol."

The bark of *Cinnamomum loureirii* is obtained from trees growing wild in southern China and Indo-China and is exported chiefly from Saigon. It occurs in the entire or unscraped form; consequently it is usually much thicker than Ceylon bark and has a quite different flavor. It is also curled into quills but these are usually of only one layer of bark and the edges frequently do not completely meet.

The U. S. P. XII requires that the bark contain not less than $2^1/_2\%$ of volatile oil. The oil contains cinnamic aldehyde and has been reported to contain eugenol and cineol.

Cassia bark or Chinese cinnamon is obtained from cultivated trees of *Cinnamomum cassia* grown in southeastern China. After collection the outer surface of the bark is partially removed by scraping or planing. The bark is then dried and forms quills similar in appearance to Saigon cinnamon. The oil of cinnamon recognized in the U. S. P. XII is obtained from leaves and twigs of this species and is required to contain "not less than 80%, by volume, of aldehydes." This is almost entirely cinnamic aldehyde and no eugenol is present. Cassia buds are also obtained from this species.

Under the terms Ground Cinnamon or Ground Cassia the Food and Drug Administration standards recognize the powder made from the barks of either *C. zeylanicum* or *C. cassia*. It must contain "not more than 5% of total ash, nor more than 2% of ash insoluble in hydrochloric acid."

---

[18] Am. Joint Committee on Horticultural Nomenclature, *Standardized Plant Names*, 1942.

9. Clove

Cloves consist of the dried flower buds of *Caryophyllus aromaticus* L.,[14] a large evergreen tree growing to a height of 30 to 40 ft. and bearing clusters of pink, fragrant flowers. The plant is native to the Molucca or Spice Islands but is cultivated in large groves on many of the East Indies and in Zanzibar, Ceylon, and Madagascar.

The clove is usually about one-half inch in length or slightly longer and is composed of a more or less cylindrical receptacle which terminates in 4 spreading, pointed sepals. The unexpanded head of the clove is composed of 4 petals curled into a spherical bud, and is partially surrounded by the toothlike sepals. When the buds have turned a red color they are collected by hand picking or by carefully beating the upper branches of the tree with poles. Having been gathered in cloths spread underneath the trees, they are immediately and carefully dried in the sun or by artificial heat. During this process they lose about half their weight and assume a brown color. The trees continue to bear one crop a year for about 25 years. Mother of clove consists of the ripened fruit of the clove tree and is much inferior to true clove as a spice.

The principal flavoring component of the clove is a volatile oil which contains 85 to 90% eugenol, some caryophyllene, vanillin, and small amounts of a number of other substances. Clove also contains about 9 to 10% of a fixed oil. The standards of the Food and Drug Administration state that cloves "contain not more than 5% of clove stems, not less than 15% of volatile ether extract, not less than 12% of quercitannic acid (calculated from the total oxygen absorbed by the aqueous extract), not more than 10% of crude fiber, not more than 7% of total ash, nor more than 0.5% of ash insoluble in hydrochloric acid."

10. Coriander

Coriander or coriander "seed" is the dried ripe fruit of *Coriandrum sativum* L., an annual herb growing to a height of about 2 ft. and having a very disagreeable odor. It is a member of the parsley family, the pink or white flowers being arranged in umbels as is the case with other members of the family. The plant grows wild in Europe and Asia Minor and is cultivated for the fruit especially in Germany and in parts of India, northern Africa, and the United States.

Since the unripe fruit has the disagreeable odor characteristic of the plant, it is allowed to ripen completely before harvesting. Then the plants are mowed, cured in the sun, dried, and threshed. The fruit is a light brown or yellow, ridged, almost spherical cremocarp, the larger ones being about the size of a small pea. The two carpels usually adhere to each other.

Coriander contains both a fixed and a volatile oil, the former present to the extent of about 20% while the latter usually runs less than 1%. The chief component of the volatile oil is *d*-linolool or coriandrol, an isomer of geraniol. The standards of the

---

[14] *Eugenia caryophyllate Thunb.* (U. S. P. XII) or *Syzygium aromaticum* (*Standardized Plant Names*, 2nd ed.).

Food and Drug Administration require that coriander contain "not more than 7% of total ash, nor more than 1.5% of ash insoluble in hydrochloric acid."

11. Cumin Seed

Cumin "seed" is the dried fruit of *Cuminum cyminum* L., a plant belonging to the parsley family. It is grown in India and China and in countries around the eastern end of the Mediterranean Sea. The fruit is a small ovoid cremocarp with bristly hairs arising from longitudinal ridges. It has a peculiar characteristic odor. The plants are harvested at maturity, threshed, and the fruits carefully cleaned and packed. The ground spice is used in curry powder and the oil for flavoring cordials. The volatile oil is present to the extent of about 2 to 4%; its characteristic component is an aldehyde, cuminal, and it also contains terpenes. The fruit also contains about 10% of a fixed oil. The standards of the Food and Drug Administration state that it contains "not more than 9.5% of total ash nor more than 1.5% of ash insoluble in hydrochloric acid, nor more than 5% of harmless foreign matter."

12. Curcuma (Turmeric)

Turmeric is the dried rhizome or bulbous root of *Curcuma longa* L., a perennial herb of the ginger family. The plant is native to southern Asia and is cultivated in China, India, and the East Indies where it is used both as a condiment and as a natural dyestuff. After the rhizomes have been dug and washed, they are boiled or scalded and then rapidly dried in the sun. They are either ovoid or pear-shaped (round turmeric) or slender elongated cylinders (long turmeric) and are deep yellow throughout. The spice contains about 5% of a volatile oil, containing alcohols and ketones, and the coloring substance curcumin. Powdered turmeric is used extensively as an ingredient in curry powder and in prepared mustard.

13. Dill Seed

Dill "seed" is the dried ripe fruit of *Anethum graveolens* L., an annual herb of the parsley family which is cultivated extensively in central Europe, England, and the United States. Not only the fruit, but also the leafy tops, are used as flavoring agents, especially in pickles and soups. The fruit is a small, oval cremocarp with two broad lateral wings. The carpels usually become separated during threshing and cleaning. Dill contains about 15% of a fixed oil and 2 to 4% of a characteristic volatile oil containing carvone, *d*-limonene, and terpenes. The Food and Drug Administration standards require that it contain "not more than 10% of total ash, nor more than 3% of ash insoluble in hydrochloric acid."

14. Fennel Seed

Fennel "seed" is the dried ripe fruit of cultivated varieties of *Foeniculum vulgare* Miller. The plant is a perennial herb of the parsley family usually growing about 3 ft. tall and bearing small yellow flowers. Fennel grows wild in the eastern Mediterranean region and is cultivated in many different countries, especially in Germany, France, Italy, and India. The fruit is a green or yellow cremocarp composed of two long, narrow, slightly curved carpels, which look like miniature bananas. The carpels are usually separated. Fennel contains up to 10% of fixed oil, but the flavoring component is a volatile oil, present to the extent of about 6%, which contains anethole, the ketone fenchone, and various terpenes. The standards of the Food and Drug Administration state that it "contains not more than 9% of total ash nor more than 2% of ash insoluble in hydrochloric acid."

15. Ginger

Ginger consists of the dried rhizome of *Zingiber officinale* Roscoe, a perennial plant somewhat resembling the common iris. Aerial stems, bearing leaves and flowers, arise from a branched, underground rhizome. The leaves are sheathlike, pale green, and tapering to a point, with distinct parallel veins. The flowers are rather small and light yellow with purple markings. Ginger has been known and used as a spice for many centuries; and although there is considerable doubt about its habitat, it seems to be indigenous to countries of southern Asia. It is now cultivated in many moist tropical regions including Japan, China, India, Indo-China, Malaya, the western coastal regions of Africa, and the East and West Indies. That grown in Jamaica is considered the best.

The plant is grown from segments of the rhizome in moist, well-cultivated plots. When the overground portion begins to die, which usually occurs somewhat less than a year after the planting, the rhizomes are dug. The method of preparation for the market varies in different localities. The outer cortical layers may be partially or completely removed by peeling or scraping and the rhizomes then washed and dried in the sun. Sometimes they are bleached by sulfur dioxide or chloride of lime before drying. In other cases the entire rhizome is treated with boiling water and dried. Lime is often used to coat the rhizomes to prevent attacks by insects and sometimes to conceal inferiorities. The color of the rhizome may vary from almost white through pale yellow to a rather deep tan depending upon the treatment.

The characteristic aroma of ginger is due to the presence of a volatile oil which occurs to the extent of about 3%. Its chief components are probably the sesquiterpene zingiberene, the terpenes *d*-camphene and phellandrene, and the alcohol zingiberol, although several other components have been reported present in small amounts. The pungency of the spice is due to an ether-soluble nonvolatile substance known as gingerol, a mixture of phenolic compounds containing the ketone zingerone.

The Food and Drug Administration provides the following standards for ginger:

Standards

"*Ginger*. The washed and dried, or decorticated and dried, rhizome of *Zingiber officinale* Roscoe. It contains not less than 42% of starch, not more than 8% of crude fiber, not more than 1% of lime (CaO), not less than 12% of cold-water extract, not more than 7% of total ash, nor more than 2% of ash insoluble in hydrochloric acid, nor less than 2% of ash soluble in cold water.

"*Jamaica Ginger.* Ginger grown in Jamaica. It contains not less than 15% of cold-water extract, and conforms in other respects to the standards for ginger.

"*Limed Ginger, Bleached Ginger.* Whole ginger coated with carbonate of calcium. It contains not more than 4% of carbonate of calcium nor more than 10% of total ash, and conforms in other respects to the standards for ginger."

The U. S. P. XII recognizes only Jamaica and African ginger.

16. Horse-radish

Horse-radish is the root of *Armoracia lapathifolia*[15] (*Radicula armoracia* (L.) Robinson), a perennial herb usually growing to a height of about 3 ft. The flowers are small and white; the fruit somewhat resembles that of mustard. The plant is probably a native of England, but is widely cultivated both in Europe and the United States.

The roots, which are usually $^1/_2$ to $1^1/_2$ in. in diameter, are covered with a brown bark, which is removed by peeling or scraping after the roots have been washed. Then the fleshy inner part of the root is chopped or grated and usually immediately packed in distilled vinegar to prevent loss of pungency and to aid in preventing discoloration.

The pungency of the root is due to a volatile oil produced by enzymatic hydrolysis of a glucoside. The glucoside has been reported to be sinigrin, yielding allyl isothiocyanate upon hydrolysis by the enzyme myrosin. Butyl sulfocyanide has also been reported as a component of the oil.

17. Marjoram (Leaf Marjoram, Sweet Marjoram)

Marjoram is the dried leaves, with or without a small proportion of flowering tops, of *Majorana hortensis* Moench., an annual herb of the mint family which is cultivated in the United States, France, Germany, and North Africa. The leaves are small, ovate, and covered with short velvety hairs which give them a whitish appearance. It is used chiefly as a seasoning for poultry and meats. The leaves contain about 1% of a volatile oil which contains terpenes and terpene alcohols. According to the standards of the Food and Drug Administration it "contains not more than 16% of total ash, not more than 4.5% of ash insoluble in hydrochloric acid, nor more than 19% of stems and harmless foreign material."

18. Mustard

Black mustard consists of the dried ripe seeds of *Brassica nigra* (L.) Koch, or of *Brassica juncea* (L.) Cosson or of varieties of these species. The seeds of the former species are sometimes known as brown or red mustard, while those of the latter are called Chinese mustard. The plants are annual herbs, usually growing to a height of 3 to 4 ft. and bearing yellow flowers. The fruit is a slender pod containing a number of small round seeds varying in color from light brown to almost black. They are widely distributed as weeds and are extensively cultivated for the seed, brown mustard being grown in central Europe, England, and various parts of the United States, and Chinese mustard being grown chiefly in China, Japan, India, and southern Russia.

---

[15] Am. Joint Committee on Horticultural Nomenclature, *Standardized Plant Names*, 1942.

White mustard (yellow mustard) consists of the dried ripe seeds of *Brassica hirta*[15] (*Brassica alba* Hooker filius or *Sinapis alba* L.), an annual herb usually growing to a height of about 2 ft. The flowers and fruit are similar in appearance to those of the black mustards except that the fruit is furnished with a long, flat beak. The seeds are somewhat larger than those of the black mustards and are pale yellow in color. The plant grows wild in many parts of the United States, but the commercial supplies of the seeds have come chiefly from central Europe and England.

TABLE 215

ANALYSES OF GENUINE MUSTARD[a]

Determination	Whole seeds, %			Flour, %			Hulls, %		
	Max.	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.
Moisture	6.82	5.88	6.37	9.50	5.09	6.96	9.12	5.36	7.48
Total ash	4.83	3.84	4.25	5.58	4.66	5.03	5.03	4.43	4.65
Water-soluble ash	0.73	0.45	0.56	0.27	0.09	0.19	2.33	0.95	1.67
Ash insoluble in HCl	0.56	0.16	0.32	0.50	0.08	0.27	0.22	0.04	0.12
Volatile ether extract	0.0	0.0	. . .	0.0	0.0	. . .	0.0	0.0	. . .
Nonvolatile ether extract	37.81	27.19	31.22	25.95	12.65	18.59	13.81	6.17	8.56
Total nitrogen	5.09	3.96	4.41	7.44	6.21	6.78	4.04	2.90	3.45
Crude fiber	6.53	4.21	5.04	3.28	1.87	2.42	18.95	10.90	15.20
"Starch" by acid hydrolysis	10.06	6.94	8.62	11.89	4.87	6.85	20.40	9.90	16.96
"Starch" by diastase	1.82	0.92	1.48	0.71	0.00	0.28	6.11	1.20	3.62
Alcohol extract	17.75	13.70	15.50	25.31	19.22	22.30	14.21	8.07	11.07

[a] A. E. Leach, *J. Am. Chem. Soc.*, **26**, 1203 (1904). A. G. Woodman, *Food Analysis.* McGraw-Hill, New York, 1941, p. 418.

Both black and white mustard seeds contain considerable quantities of fixed oil, usually 25 to 35%. This oil has nothing to do with the flavor of the mustard, being a bland, almost tasteless oil, and is sometimes used in the refined state as a salad oil. It is usually removed by expression before mustard products are prepared. The oils which are responsible for the flavor, pungency, and vesicant properties of mustard do not exist as such in the seed but are produced as a result of an enzymatic hydrolysis which occurs when the seeds are macerated in warm water. The enzyme, myrosin, is found in both black and white mustard seeds. In black mustard seed it breaks down the glucoside, sinigrin, forming glucose, potassium hydrogen sulfate, and allyl isothiocyanate, the characteristic volatile oil. In white mustard seed the enzyme breaks down the glucoside, sinalbin, forming glucose, sinapine hydrogen sulfate, and acrinyl isothiocyanate, which is a nonvolatile pungent oil.

The standards of the Food and Drug Administration provide that both black and white mustard seed "contain not more than 5% of total ash nor more than 1.5% of ash insoluble in hydrochloric acid"; that black mustard seed "yield 0.6% of volatile mustard oil (calculated as allyl isothiocyanate)" and that white mustard seed "contain no appreciable amount of volatile oil." The various products prepared from mustard seed are defined as follows:

Standards

"*Ground Mustard Seed, Mustard Meal.* Unbolted, ground mustard seed, conforming to the standards for mustard seed.

"*Mustard Cake.* Ground mustard seed, mustard meal, from which a portion of fixed oil has been removed.

"*Mustard Flour, Ground Mustard, 'Mustard.'* The powder made from mustard seed with the hulls largely removed and with or without the removal of a portion of the fixed oil. It contains not more than 1.5% of starch, nor more than 6% of total ash.

"*Prepared Mustard.* A paste composed of a mixture of ground mustard seed and/or mustard flour and/or mustard cake, with salt, a vinegar, and with or without sugar and/or dextrose, spices, or other condiments. In the fat-, salt-, and sugar-free solids it contains not more than 24% of carbohydrates, not more than 12% of crude fiber, nor less than 5.6% of nitrogen, the carbohydrates being calculated as starch."

19. Nutmeg and Mace

Nutmeg consists of the dried, ripe seed of *Myristica fragrans* Houtt., the seed coat or testa having been removed. Mace consists of the dried arillus of the same species. The plant is a large spreading evergreen tree which is native to the Molucca Islands. The staminate and pistillate flowers, which are usually found on separate trees, are both small and yellow in color. The fruit, borne only by the female trees, is round to ovoid, fleshy, rather fibrous, and when ripe is golden yellow and has a deep longitudinal cleft. The tree grows wild in the Banda Islands of the East Indies and is cultivated in many of the East Indies and in Malaya, Ceylon, and the West Indies.

The cultivated trees are grown in large orchards from seeds or nursery seedlings. At the age of 6 to 7 years the trees begin to bear flowers and fruit and the sex of the tree can then be determined. Most of the male trees are then removed and replaced with seedlings. The female trees bear fruit constantly so that two and sometimes three crops are harvested every year, and they continue to bear for about 60 years. At maturity the fruit splits open revealing the seed, which is covered by a scarlet, net-like arillus. The fruit is then gathered by hand or by long poles equipped with small baskets and hooks. After the fruit pulp or husk has been removed by beating, the arillus is carefully removed by hand and dried. During the drying process it changes from scarlet to a golden-brown color and is then packed and exported as mace. The seeds are slowly and carefully dried by heating until the kernel rattles within the seed coat when shaken. The seed coats are then carefully broken by a mallet and removed; the kernels are graded and sometimes coated with lime, and exported. Nutmegs are usually oval and about an inch in length and slightly over half an inch in diameter, although the size varies considerably. They are brown in color and the external surface is covered with a network of shallow furrows.

Nutmeg and mace both contain relatively large amounts of fixed oil, nutmeg containing about 35 to 40% and mace about 20 to 25%. The chief component of this oil is trimyristin; it contains small amounts of the glyceryl esters of palmitic, oleic, and linolenic acids and some unsaponifiable matter including myristicin. The flavoring components of mace and nutmeg are volatile oils which are similar but not identical in composition. They both contain myristicin and various terpenes.

The standards of the Food and Drug Administration for nutmeg require that it contain "not less than 25% of nonvolatile ether extract, not more than 10% of crude fiber, not more than 5% of total ash, nor more than 0.5% of ash insoluble in hydrochloric acid." Mace is required to contain "not less than 20% nor more than 30% of nonvolatile ether extract, not more than 10% of crude fiber, not more than 3% of total ash, nor more than 0.5% of ash insoluble in hydrochloric acid." The dried seed and arillus of *Myristica argentea* Warb. are recognized as Macassar, Papua, Male or Long Nutmeg and as Macassar or Papua Mace, respectively. These are inferior in aroma and flavoring properties to true nutmeg and mace.

20. Parsley

Both the leaves and the dried ripe fruit of *Petroselinum crispum latifolium*[15] (*P. hortense* Hoffm. or *P. sativum* Hoffm.) are used as condiments. The plant is the common garden parsley which is extensively cultivated in the United States and Europe. It is a biennial herb of the parsley family. The fruit is an ovoid cremocarp, the carpels usually being separated. The fruit contains about 6 to 7% of a volatile oil, the chief component of which is apiol.

21. Pepper

Black pepper consists of the dried, unripe fruit of *Piper nigrum* L., a perennial vine with rather large fleshy leaves, small white flowers and a small spherical fruit borne on spikes. On each spike there are usually 20 to 40 berries which are at first green but upon ripening turn red and finally yellowish; a single vine usually bears 20 or more fruit spikes. Those vines which are uncared for may trail along the ground, growing to a length of 30 ft. or more and becoming quite woody. Practically all the pepper of commerce, however, comes from pepper gardens where the plants are cultivated and kept cut back to about 8 to 10 ft. and are supported by posts or, more often, trees which are planted especially to support and shade the vine. The pepper vine is indigenous to certain provinces of India but is extensively cultivated in India, the East Indies, the Malay states, and to some extent in the West Indies and northern South America.

As soon as the oldest fruits on the spike begin to turn red in color, the entire spike is picked. There are usually two crops each year and a vine may bear good fruit for 15 years or longer. The spikes are dried either in the sun or over smoky fires, during which process the berry becomes wrinkled and dark in color. After drying, the berries are separated from the stalk, cleaned, graded, and packed for exportation.

There are a number of commercial varieties of which Singapore and Tellicherry are generally considered the best.

Pepper contains about 1 to 3% of volatile oil but the component most responsible for its pungency seems to be the alkaloids which are present. It contains the crystalline alkaloid piperine, the liquid alkaloid piperidine, and a pungent substance known as chavicine. There is very little or no true fixed oil but the alkaloids cause an appreciable nonvolatile ether-soluble extract. The Food and Drug Administration standards for black pepper are as follows: "It contains not less than 6.75% of nonvolatile ether extract, not less than 30% starch, not more than 7% of total ash, nor more than 1.5% of ash insoluble in hydrochloric acid."

White pepper is obtained from the same plant as black pepper and consists of the dried, mature fruit from which the outer coating or the outer and inner coatings have been removed. This is usually accomplished by friction which is sometimes preceded by a preliminary fermentation or soaking. It is less pungent than the black. The standards of the Food and Drug Administration provide that it contain "not less than 7% of nonvolatile ether extract, not less than 52% of starch, not more than 5% of crude fiber, not more than 3.5% of total ash, nor more than 0.3% of ash insoluble in hydrochloric acid."

Long pepper is the dried fruit of *Piper longum* L., a shrub grown to some extent in the Philippine Islands and in Java as a substitute for pepper. The entire unripe spike is used.

Red pepper is the dried ripe fruit of various species of the genus *Capsicum*, plants not at all closely related to the true pepper discussed above. According to the standards of the Food and Drug Administration, cayenne pepper is "the dried ripe fruit of *Capsicum frutescens* L., *C. baccatum* L., or some other small-fruited species of *Capsicum*." Among these other species Youngken[16] mentions certain varieties of *C. annum* L. In the latest edition of *Standardized Plant Names*, the only species of *Capsicum* which is recognized is *C. frutescens*, several varieties being listed. That there is some difference of opinion concerning the classification of the genus *Capsicum* is conceded, however, by the following note:[17] "The taxonomic authorities of this edition of S. P. N. maintain that *Capsicum annum* is indistinguishable from *C. frutescens*, remanding the former to synonymy. Dr. Youngken maintains that there are definite histological (as well as pharmaceutical) differences between the two."

The plant is a perennial or annual shrub bearing bright red, pungent fruit, the size of which varies considerably depending upon the variety or species from which it is taken. It is cultivated extensively in the southern part of the United States and in Mexico, and other tropical countries, particularly in east Africa.

The pungent principle of the fruit is known as capsaicin. The standards of the Food and Drug Administration require that cayenne pepper contain "not less than 15% of nonvolatile ether extract, not more than 1.5% starch, not more than 28% crude fiber, not more than 8% of total ash, not more than 1.25% of ash insoluble in hydrochloric acid."

---

[16] H. W. Youngken, *Textbook of Pharmacognosy.* Blakiston, Philadelphia, 1943.

[17] Am. Joint Committee on Horticultural Nomenclature, *Standardized Plant Names*, 1942, p. 177.

Paprika is the dried ripe fruit of certain varieties of *Capsicum annuum* L. The following Food and Drug Administration standards indicate the principal varieties of paprika which are used:

Standards

"*Hungarian Paprika.* Paprika having the pungency and flavor characteristic of that grown in Hungary.

"(*a*) Rosenpaprika, rosapaprika, rose paprika, is Hungarian paprika prepared by grinding specially selected pods of paprika, from which the placentae, stalks, and stems have been removed. It contains no more seeds than the normal pods, not more than 18% of nonvolatile ether extract, not more than 23% of crude fiber, not more than 6% of total ash, nor more than 0.4% of ash insoluble in hydrochloric acid.

"(*b*) Koenigspaprika, king's paprika, is Hungarian paprika prepared by grinding whole pods of paprika without selection, and includes the seed and stems naturally occurring with the pods. It contains not more than 18% of nonvolatile ether extract, not more than 23% of crude fiber, not more than 6.5% of total ash, nor more than 0.5% of ash insoluble in hydrochloric acid.

"*Pimenton, Pimiento, Spanish Paprika.* Paprika having the characteristics of that grown in Spain. It contains not more than 18% of nonvolatile ether extract, nor more than 21% of crude fiber, not more than 8.5% of total ash, nor more than 1% of ash insoluble in hydrochloric acid."

22. Saffron

Saffron consists of the dried stigma of *Crocus sativus* L., a perennial plant of the iris family closely related to the common flower-garden crocus. Saffron has been used extensively in the past both as a natural dyestuff and as a spice, but is used very infrequently now. The plant is native to southern Europe and is cultivated chiefly in Spain, France, and Italy. The stigmas are collected by hand and dried by heat. The enormous amount of labor involved in collection makes the material quite expensive, and it has been subject to much adulteration. There are usually three dark red, trumpet-shaped stigmas attached to a yellow style. Saffron contains small amounts of fixed and volatile oils, the glycoside picrocrocin, and the coloring principles crocin and crocetin. The standards of the Food and Drug Administration require that it contain "not more than 10% of yellow styles and other foreign matter, not more than 14% of volatile matter when dried at 100° C., not more than 7.5% of total ash, nor more than 1% of ash insoluble in hydrochloric acid."

23. Sage

Sage is the dried leaves of *Salvia officinalis* L., a perennial shrub of the mint family usually growing to a height of 1 to 2 ft. It is cultivated in various parts of Europe and the United States as a garden herb. The leaves and stems of the plant are covered with fine, silky, grayish hairs which give them a grayish-green color. It is used extensively as a flavor, especially for sausage and other meat products and for fowl. Sage contains up to $2^1/_2$% of a volatile oil, the characteristic component of which is the ketone, thujone. The oil also contains a high percentage of borneol and a number of other minor components. The Food and Drug Administration standards require that sage contain "not more than 12% of stems (excluding petioles) and other foreign material."

24. Savory (Summer Savory)

Savory consists of the dried leaves and flowering tops of *Satureia hortensis* L., an annual herb of the mint family. Like sage, the entire plant is pubescent. It is cultivated extensively in the Mediterranean region, and is used not only in the dried but also in the fresh state for flavoring soups, sauces, and gravies. Savory contains about 1% of a volatile oil, the chief components of which are carvacrol and other phenols and terpenes.

25. Tarragon

Tarragon is the dried leaves and flowering tops of *Artemisia dracunculus* L., an aromatic herb closely related to wormwood but with a pleasant aniselike odor and taste. The plant is native to Siberia but is now cultivated in France and other European countries and to a small extent in the United States. It contains about 10% of a volatile oil containing methyl chavicol.

26. Thyme

Thyme is the dried leaves and flowering tops of *Thymus vulgaris* L., an annual herb of the mint family which has small hairy leaves and purple flowers. It is cultivated extensively in central Europe. Thyme contains about 2% of a volatile oil the chief components of which are the phenols thymol and carvacrol. The Food and Drug Administration standards require that thyme contain "not more than 14% of total ash, nor more than 4% of ash insoluble in hydrochloric acid."

II. FLAVORING EXTRACTS

A flavoring extract is defined[18] as "A solution in ethyl alcohol of proper strength of the sapid and odorous principles derived from an aromatic plant, or parts of the plant, with or without its coloring matter, conforming in name to the plant used in its preparation." This definition applies only to those extracts which are intended solely for food purposes. Similar preparations described in the United States Pharmacopoeia and the National Formulary for medicinal purposes should not be confused with them, although formulas from these books are sometimes used for preparing certain flavoring extracts of superior quality.

The flavoring extract may be prepared by maceration or percolation of the appropriate plant or plant part with ethyl alcohol of suitable concentration. This is the method usually employed in preparing such extracts as those of vanilla, ginger, and tonka. More often the extract is made by dissolving an essential oil obtained from the plant by distillation or expression in ethyl alcohol of suitable concentration. Imitation extracts are prepared from flavoring principles made synthetically or obtained from plants other than the one from which the extract derives its name. In certain cases, as in the preparation of vanilla extract, sugar, dextrose, or glycerol may be added; but if coloring matter other than the natural coloring principles of the appropriate plant are added, information to this effect must be given on the label.

---

[18] U. S. Dept. Agr., Food and Drug Admin., Service and Regulatory Announcements, Food and Drug No. 2 (1936).

The Food and Drug Administration has published standards for a number of essential oils, from which flavoring extracts may be prepared, and for flavoring extracts of almond, anise, celery seed, cinnamon, Ceylon cinnamon, clove, ginger, lemon, nutmeg, orange, peppermint, rose, savory, spearmint, star anise, sweet basil, sweet marjoram, thyme, tonka, vanilla, and wintergreen. Extracts of ginger, tonka, and vanilla are to be prepared directly from the appropriate plant part. Extracts of almond, anise, cinnamon, Ceylon cinnamon, clove, nutmeg, rose, star anise, and wintergreen are to be made by solution of the appropriate essential oil. The other extracts may be prepared by either method.

Vanilla and lemon extracts are by far the most frequently used and provide examples of each method of preparation.

1. Vanilla Extract

This extract is prepared directly from the vanilla bean with or without the addition of dextrose, sugar, or glycerol. In each 100 ml. it contains the soluble matters from not less than 10 g. of the vanilla bean.

Vanilla bean is the cured, full-grown, unripe fruit of *Vanilla planifolia* Andrews (*Vanilla fragrans* Salisb.) or *V. tahitensis* Moore. The former plant is a perennial, epiphytic vine of the orchid family. The succulent stem attaches itself to the trunks and branches of trees by means of aerial roots and bears fleshy, oval leaves and pale yellow flowers. The fruit is a long bean containing a brown pulp in which is imbedded large numbers of very small, hard, black seeds. It is native to eastern Mexico where cultivated vines now produce the best commercial beans. It is also cultivated in the East and West Indies, Madagascar, and other tropical regions. Bourbon or Réunion vanilla comes from the Isle of Réunion. *V. tahitensis* is grown in Tahiti and Hawaii and produces beans having a distinctly inferior flavor to that of the Mexican beans.

The vanilla plant is dioecious and in Mexico artificial pollination is used in order to obtain a large crop of fruit. Natives carry the pollen grains from the male to the female flowers on feathers. When the fruits have reached maturity and are beginning to turn yellow, they are picked, placed in piles, and allowed to ferment. After an alternate sweating and drying process, which covers a period of several weeks and during which the beans become dark brown in color, they are dried in the sun, given a coating of oil, tied in bundles, and packed for export. The surface of the beans frequently becomes covered with small crystals of vanillin which has been produced during the curing process.

For the preparation of the extract, the beans, cut into small pieces and bruised, are macerated in and then percolated with diluted alcohol. It has been found that if alcohol of less than about 45% strength is used, gelatinous materials are extracted which clog the percolators. Frequently sugar or glycerol is added during the percolation process. More highly colored extracts are usually obtained when glycerol is used in the menstruum.

TABLE 216

COMPOSITION OF PURE VANILLA EXTRACTS[a]

Kind of bean	Vanillin, g. in 100 ml.	Normal lead number	Color insoluble in amyl alcohol, %	Acidity, ml. 0.1 N alkali per 100 ml.	Ash, g. in 100 ml.	Alkalinity of ash, ml. 0.1 N acid per 100 ml.
Mexican:						
Min.	0.15	0.47	19.0	42	0.297	36
Max.	0.20	0.68	24.4	52	0.422	53
Av.	0.17	0.58	21.2	46	0.359	45
Bourbon:						
Min.	0.13	0.44	21.3	35	0.263	34
Max.	0.22	0.63	30.3	51	0.373	52
Av.	0.18	0.52	26.6	40	0.317	40
Seychelles:						
Min.	0.16	0.45	22.7	35	0.251	33
Max.	0.21	0.60	29.4	42	0.361	47
Av.	0.19	0.51	25.6	39	0.293	39
Madagascar:						
Min.	0.16	0.40	23.2	42	0.220	34
Max.	0.30	0.63	30.3	47	0.326	47
Av.	0.22	0.50	26.8	45	0.284	39
Comores:						
Min.	0.12	0.40	20.4	34	0.229	31
Max.	0.31	0.74	30.3	47	0.432	55
Av.	0.18	0.59	26.7	40	0.333	45
South American:						
Min.	0.19	0.49	20.0	44	0.305	38
Max.	0.23	0.58	29.4	52	0.344	42
Av.	0.21	0.52	23.3	49	0.325	40
Java:						
Min.	0.22	0.44	32.2	45	0.290	38
Max.	0.24	0.61	35.7	52	0.349	49
Av.	0.23	0.50	34.5	48	0.311	42
Tahiti:						
Min.	0.11	0.44	16.0	30	0.221	30
Max.	0.17	0.56	22.0	33	0.288	37
Av.	0.13	0.50	18.0	31	0.254	33
All analyses:						
Min.	0.11	0.40	16.0	30	0.220	29
Max.	0.31	0.74	35.7	52	0.432	58
General av.	0.19	0.54	25.5	42	0.319	42

[a] A. L. Winton and K. G. B. Winton, *Structure and Composition of Foods*. Vol. IV, Wiley, New York, 1939, p. 314.

The cured bean contains as its chief flavoring principle the phenol, vanillin or methylprotocatechuic aldehyde, which is found in a number of other plants and which can be synthesized quite economically from eugenol or coniferin. Other flavoring substances are present in small amounts, however, so that vanillin alone does not provide tonka. More often the extract is made by dissolving an essential oil obtained from the plant by distillation or expression in ethyl alcohol of suitable concentration. Imitation extracts are prepared from flavoring principles made synthetically or obtained from plants other than the one from which the extract derives its name. In certain cases, as in the preparation of vanilla extract, sugar, dextrose, or glycerol may be added; but if coloring matter other than the natural coloring principles of the appropriate plant are added, information to this effect must be given on the label.

The Food and Drug Administration has published standards for a number of essential oils, from which flavoring extracts may be prepared, and for flavoring extracts of almond, anise, celery seed, cinnamon, Ceylon cinnamon, clove, ginger, lemon, nutmeg, orange, peppermint, rose, savory, spearmint, star anise, sweet basil, sweet marjoram, thyme, tonka, vanilla, and wintergreen. Extracts of ginger, tonka, and vanilla are to be prepared directly from the appropriate plant part. Extracts of almond, anise, cinnamon, Ceylon cinnamon, clove, nutmeg, rose, star anise, and wintergreen are to be made by solution of the appropriate essential oil. The other extracts may be prepared by either method.

Vanilla and lemon extracts are by far the most frequently used and provide examples of each method of preparation.

1. Vanilla Extract

This extract is prepared directly from the vanilla bean with or without the addition of dextrose, sugar, or glycerol. In each 100 ml. it contains the soluble matters from not less than 10 g. of the vanilla bean.

Vanilla bean is the cured, full-grown, unripe fruit of *Vanilla planifolia* Andrews (*Vanilla fragrans* Salisb.) or *V. tahitensis* Moore. The former plant is a perennial, epiphytic vine of the orchid family. The succulent stem attaches itself to the trunks and branches of trees by means of aerial roots and bears fleshy, oval leaves and pale yellow flowers. The fruit is a long bean containing a brown pulp in which is imbedded large numbers of very small, hard, black seeds. It is native to eastern Mexico where cultivated vines now produce the best commercial beans. It is also cultivated in the East and West Indies, Madagascar, and other tropical regions. Bourbon or Réunion vanilla comes from the Isle of Réunion. *V. tahitensis* is grown in Tahiti and Hawaii and produces beans having a distinctly inferior flavor to that of the Mexican beans.

The vanilla plant is dioecious and in Mexico artificial pollination is used in order to obtain a large crop of fruit. Natives carry the pollen grains from the male to the female flowers on feathers. When the fruits have reached maturity and are beginning to turn yellow, they are picked, placed in piles, and allowed to ferment. After an alternate sweating and drying process, which covers a period of several weeks and during which the beans become dark brown in color, they are dried in the sun, given a coating of oil, tied in bundles, and packed for export. The surface of the beans frequently becomes covered with small crystals of vanillin which has been produced during the curing process.

For the preparation of the extract, the beans, cut into small pieces and bruised, are macerated in and then percolated with diluted alcohol. It has been found that if alcohol of less than about 45% strength is used, gelatinous materials are extracted which clog the percolators. Frequently sugar or glycerol is added during the percolation process. More highly colored extracts are usually obtained when glycerol is used in the menstruum.

TABLE 216

COMPOSITION OF PURE VANILLA EXTRACTS[a]

Kind of bean	Vanillin, g. in 100 ml.	Normal lead number	Color insoluble in amyl alcohol, %	Acidity, ml. 0.1 N alkali per 100 ml.	Ash, g. in 100 ml.	Alkalinity of ash, ml. 0.1 N acid per 100 ml.
Mexican:						
Min.	0.15	0.47	19.0	42	0.297	36
Max.	0.20	0.68	24.4	52	0.422	53
Av.	0.17	0.58	21.2	46	0.359	45
Bourbon:						
Min.	0.13	0.44	21.3	35	0.263	34
Max.	0.22	0.63	30.3	51	0.373	52
Av.	0.18	0.52	26.6	40	0.317	40
Seychelles:						
Min.	0.16	0.45	22.7	35	0.251	33
Max.	0.21	0.60	29.4	42	0.361	47
Av.	0.19	0.51	25.6	39	0.293	39
Madagascar:						
Min.	0.16	0.40	23.2	42	0.220	34
Max.	0.30	0.63	30.3	47	0.326	47
Av.	0.22	0.50	26.8	45	0.284	39
Comores:						
Min.	0.12	0.40	20.4	34	0.229	31
Max.	0.31	0.74	30.3	47	0.432	55
Av.	0.18	0.59	26.7	40	0.333	45
South American:						
Min.	0.19	0.49	20.0	44	0.305	38
Max.	0.23	0.58	29.4	52	0.344	42
Av.	0.21	0.52	23.3	49	0.325	40
Java:						
Min.	0.22	0.44	32.2	45	0.290	38
Max.	0.24	0.61	35.7	52	0.349	49
Av.	0.23	0.50	34.5	48	0.311	42
Tahiti:						
Min.	0.11	0.44	16.0	30	0.221	30
Max.	0.17	0.56	22.0	33	0.288	37
Av.	0.13	0.50	18.0	31	0.254	33
All analyses:						
Min.	0.11	0.40	16.0	30	0.220	29
Max.	0.31	0.74	35.7	52	0.432	58
General av.	0.19	0.54	25.5	42	0.319	42

[a] A. L. Winton and K. G. B. Winton, *Structure and Composition of Foods*. Vol. IV, Wiley, New York, 1939, p. 314.

The cured bean contains as its chief flavoring principle the phenol, vanillin or methylprotocatechuic aldehyde, which is found in a number of other plants and which can be synthesized quite economically from eugenol or coniferin. Other flavoring substances are present in small amounts, however, so that vanillin alone does not provide the true flavor of the extract. A number of complex resinous materials are also present and, being soluble in the menstruum, are found in the finished extract. While these have not been identified, they give certain characteristic qualitative tests; hence their absence in artificial extracts can be shown.

Adulterated extracts frequently contain coumarin, the principal flavoring material in tonka bean, the seed of *Dipteryx odorata* Aubl. (*Coumarouna odorata* Aublet). Extract of tonka has the same general physical appearance as vanilla extract and a similar odor; it is frequently used as an adulterant. Wholly synthetic vanilla extracts are also sold, being made from crystalline vanillin and coumarin and colored with caramel or coal-tar dye.

Detailed procedures for the analysis of vanilla extract may be found in *Methods of Analysis*, of the A. O. A. C. Table 216 shows the results of analyses of genuine vanilla extracts prepared from beans from various sources. Winton[19] and Woodman[20] also give tables showing the composition of extracts prepared by various methods. In addition to the usual determinations made in proximate analysis, methods are given for the gravimetric and colorimetric determination of vanillin and for the determination of coumarin, lead number, vanilla resins, and color insoluble in amyl alcohol. The lead number is a measure of the amount of lead in the precipitate after precipitation with neutral lead acetate solution and is an empirical value. It varies less in genuine vanilla extracts, however, than most of the other values; and the figure, considered in conjunction with the physical appearance of the lead acetate precipitate, will frequently clearly indicate adulteration. The lead number of tonka extract is much lower than that of vanilla extract, while the lead number of a purely synthetic *extract* containing no plant extractive would be even lower. The percentage of total color insoluble in amyl alcohol is valuable in detecting artificially colored extracts. Although a considerable variation in values is to be expected, it seems almost certain that a genuine vanilla extract will contain from 0.10 to 0.35% vanillin and no coumarin, have a lead number (Winton) of not less than 0.40 nor more than 0.80, and show no more than 36% color insoluble in amyl alcohol.

2. Lemon Extract

Although lemon extract was probably prepared in the past from the fresh lemon peel, practically all the extract on the market now is prepared by dissolving lemon oil in alcohol of the proper strength. The Food and Drug Administration standards recognize extracts made by either method and state that lemon extract "contains not less than 5% by volume of oil of lemon." Oil of lemon is the volatile oil obtained by expression, without the aid of heat, from the fresh peel of the fruit of *Citrus medica* variety *Limon* L. (*Citrus limonia* Osbeck). Most of the oil used in this country is produced in California and is expressed by machines. In the past considerable quantities of hand-pressed oil have been imported from Italy and Sicily. It is a light yellow or yellowish-green liquid containing about 90% of the terpene *d*-limonene, about 5% of the aldehyde citral, and a number of other terpenes in small amounts. Citral is the principal flavoring material but the minor components add to and modify the flavor somewhat.

---

[19] A. L. Winton and K. G. B. Winton, *Structure and Composition of Foods.* Vol. IV, Wiley, New York, 1939.

[20] A. G. Woodman, *Food Analysis.* McGraw-Hill, New York, 1941.

The standards of the Food and Drug Administration also recognize a "Terpeneless Extract of Lemon" which can be prepared by shaking oil of lemon with dilute alcohol or by dissolving an oil of lemon from which all or nearly all of the terpenes have been removed (recognized as "Terpeneless Oil of Lemon") in dilute alcohol. "It contains not less than 0.2% by weight of citral derived from oil of lemon." True oil of lemon requires rather concentrated alcohol for solution; but citral, the principal flavoring ingredient, is soluble in dilute alcohol. Since the oil is much less expensive than alcohol, the terpeneless extract can be sold much more cheaply than the true extract, but it lacks the modification of the flavor introduced by some of the minor terpene components. Almost all lemon extracts are colored artificially since the public expects a bright yellow-colored extract. The presence of artificial colors must, of course, be indicated on the label.

Methods of Analysis of the A. O. A. C. contains methods for the analysis of lemon extract. Oil of lemon can be determined by polarization or by precipitation, centrifuging, and direct measurement. Alcohol is determined in the usual way after absorbing the volatile oil with magnesium carbonate. Citral is determined colorimetrically.

3. Synthetic Flavors

Since the use of the natural flavoring oils and of flavoring extracts prepared from them is prohibitively expensive for the preparation of certain foods and beverages, a large number of artificial flavors are produced and sold. These are used in large quantities by producers of soft drinks, candies, ice cream, and various other confections, and are also bottled and sold for use in the home. They imitate more or less closely the taste and aroma of the natural flavoring principles.

A few natural flavors can be simulated by the use of simple esters; the bananalike odor of amyl acetate is well known. But most of the artificial flavors are mixtures of various synthetic esters, aldehydes, ketones, and alcohols. Sometimes small amounts of chloroform and of various organic acids, as tartaric, citric, succinic, and benzoic acids, are also used. These mixtures are usually dissolved in ethyl alcohol with or without the addition of small amounts of glycerol, and are sold in quite concentrated form.

As examples of synthetic or imitation flavors the following formulas[21] for artificial fruit oils are illustrative.

---

[21] F. Fiene and S. Blumenthal, *Handbook of Food Manufacture.* Chemical Pub. Co., New York, 1938 p. 153.

Apple Oil

Chloroform	1 oz.
Ethyl butyrate	10 oz.
Ethyl valerianate	5 oz.
Ethyl salicylate	2 oz.
Amyl butyrate	1 oz.
Glycerol	4 oz.
Alcohol	6 pt

Banana Oil

Ethyl butyrate	10 oz.
Amyl acetate	10 oz.
Alcohol	6 pt.

Cherry Oil

Chloroform	5 oz.
Ethyl benzoate	15 oz.
Ethyl acetate	15 oz.
Oil of bitter almond	2 oz.
Amyl acetate	1 oz.
Alcohol	6 pt.

Pear Oil

Ethyl nitrite	5 oz.
Amyl acetate	10 oz.
Glycerol	10 oz.
Alcohol	6 pt

Strawberry Oil

Ethyl butyrate	5 oz.
Ethyl formate	1 oz.
Ethyl nitrite	1 oz.
Ethyl salicylate	1 oz.
Ethyl acetate	5 oz.
Amyl acetate	3 oz.
Amyl butyrate	2 oz.
Glycerol	2 oz.
Alcohol	6 pt.

Pineapple Oil

Ethyl butyrate	5 oz.
Amyl butyrate	10 oz.
Chloroform	1 oz.
Acetaldehyde	1 oz.
Glycerol	3 oz.
Alcohol	6 pt.

These oils may be diluted as much as 1 part in 8 parts with a hydroalcoholic menstruum of suitable strength before being used as flavors.

III. VINEGAR

The standards of the Food and Drug Administration define the various types of vinegars as follows:

Standards:

"*Vinegar, Cider Vinegar, Apple Vinegar.* The product made by the alcoholic and subsequent acetous fermentations of the juice of apples. It contains, in 100 cc. (20° C.), not less than 4 g. of acetic acid.

"*Wine Vinegar, Grape Vinegar.* The product made by the alcoholic and subsequent acetous fermentations of the juice of grapes. It contains, in 100 cc. (20° C.), not less than 4 g. of acetic acid.

"*Malt Vinegar.* The product made by the alcoholic and subsequent acetous fermentations, without distillation, of an infusion of barley malt or cereals whose starch has been converted by malt. It contains, in 100 cc. (20° C.), not less than 4 g. of acetic acid.

"*Sugar Vinegar.* The product made by the alcoholic and subsequent acetous fermentations of sugar sirup, molasses, or refiners sirup. It contains, in 100 cc. (20° C.), not less than 4 g. of acetic acid.

"*Glucose Vinegar.* The product made by the alcoholic and subsequent acetous fermentations of a solution of glucose. It is dextrorotatory and contains, in 100 cc. (20° C.), not less than 4 g. of acetic acid.

"*Spirit Vinegar, Distilled Vinegar, Grain Vinegar.* The product made by the acetous fermentation of dilute distilled alcohol. It contains, in 100 cc. (20° C.), not less than 4 g. of acetic acid."

It should be noted that the unqualified term "vinegar" is used only to designate cider or apple vinegar. For many years cider vinegar was the only kind of vinegar sold in this country; so it is proper to consider that, when the consumer asks for vinegar, he means cider vinegar unless he specifies some other type. All of the types defined above are products made by a fermentation process; hence diluted acetic acid, whether flavored or not, does not meet the definition for vinegar.

Vinegar can be prepared by allowing the alcoholic liquid to stand for several months in casks or barrels, exposed to the air. The so-called generator method is quicker and can be controlled more accurately; it is the method most frequently used. A tall, cylindrical tank, or generator, is filled with wood shavings or other fibrous materials which have been impregnated with a culture of *Mycoderma aceti.* The alcoholic liquid is allowed to trickle down through the generator, while air is being forced up through it from the bottom. The organisms, utilizing the oxygen of the air, convert the alcohol into acetic acid quite rapidly under these conditions. Various other products of fermentation, such as other organic acids, furfural, acetylmethylcarbinol and aldehydes, are formed in small amounts; and they, along with some unfermented sugar and glycerol from the alcoholic fermentation, give the product its characteristic flavor and aroma.

Procedures for the analysis of vinegar may be found in *Methods of Analysis* of the A. O. A. C. By comparing values obtained with known normal values from such determinations as total solids, ash, soluble and insoluble ash, alkalinity of ash, soluble and insoluble phosphoric acid, total acid, volatile and nonvolatile acids, reducing substances both before and after inversion, and alcohol, the genuineness of a sample can often be proved or disproved. Methods by which the genuineness of a vinegar can be established are also given by Jacobs. The glycerol determination, tests for mineral acids, and the determination of volatile reducing substances, chiefly acetylmethylcarbinol, are valuable in proving whether a sample is a product of acetous fermentation or is purely artificial.

TABLE 217

COMPOSITION OF VINEGAR[a]

Determination	Av.	Max.	Min.
Total acid as acetic acid, %	4.94	7.96	3.29
Total solids, %	2.54	4.52	1.37
Nonsugar solids, %	1.90	2.89	1.26
Reducing sugars in solids, %	19.6	45.0	5.6
Total ash, %	0.367	0.52	0.20
Alkalinity of water-soluble ash, ml.	35.7	56.0	21.5
Ash in nonsugar solids, %	18.8	26.5	11.2
Soluble phosphoric acid (mg. P_2O_5)	17.3	39.9	6.7
Insoluble phosphoric acid (mg. P_2O_5)	12.0	32.0	4.3
Total phosphoric acid (mg. P_2O_5)	29.3	64.2	15.1
Polarization (direct), ° V.	−1.46	−3.6	−0.2
Polarization (invert), ° V.	−1.69	−3.1	±0.0

[a] R. W. Balcom, U. S. Dept. Agr., Bur. Chem., *Bull.* **132** (1910). M. B. Jacobs, *Chemical Analysis of Foods and Food Products.* Van Nostrand, New York, 1938, p. 349.

TABLE 218

CIDER VINEGAR ANALYSES[a]

Determination	Min.	Max.	Av.
Specific gravity (15.6° C.)	1.0126	1.0275	1.0177
Acidity as acetic acid, g. per 100 ml.	3.24	9.96	5.21
Nonvolatile acid as lactic, g. per 100 ml.	0.05	0.30	0.18
Alcohol by volume, g. per 100 ml.	0.03	2.00	0.35
Glycerol, g. per 100 ml.	0.23	0.46	0.30
Total solids, g. per 100 ml.	1.20	4.45	2.40
Reducing sugars as invert sugar, g. per 100 ml.	0.11	1.12	0.47
Volatile reducing substances as invert sugar, g. per 100 ml.	0.14	0.34	0.20
Nonsugar solids, g. per 100 ml.	1.00	2.90	1.90
Pentosans, g. per 100 ml.	0.08	0.22	0.16
Formic acid, g. per 100 ml.	0.0003	0.009	0.003
Volatile esters as ethyl acetate, g. per 100 ml.	0.30	0.91	....
Total ash, g. per 100 ml.	0.20	0.57	0.38
Soluble ash, g. per 100 ml.	0.17	0.51	0.34
Phosphoric acid in soluble ash, g. per 100 ml.	0.007	0.040	0.017
Phosphoric acid in insoluble ash, g. per 100 ml.	0.004	0.032	0.012
Alkalinity of soluble ash, ml. 0.1 *N* acid	21.5	56.0	35.0
Polarization in 200-mm. tube, ° V.	−0.2	−3.6	−1.4
Sugars in total solids, %	5.3	43.3	20.2
Total ash in nonsugar solids, %	12.0	30.0	19.0

[a] R. O. Brooks, *Critical Studies in the Legal Chemistry of Foods*. Chemical Catalog Co., New York, 1927, p. 84. A. G. Woodman, *Food Analysis*. McGraw-Hill, New York, 1941, p. 440.

Most of the adulterations of vinegar consist of the addition of other types of vinegar to cider vinegar or a complete substitution of some other type for cider vinegar. By a careful consideration of all the analytical constants, these can usually be detected rather easily. Some of the constants of cider vinegar may be matched in the adulterated sample, but it rarely happens that all of them can be matched.

IV. MAYONNAISE AND SALAD DRESSINGS

Although mayonnaise and similar types of salad dressings have been made and used in the home in this country for many years, it has been only recently that commercial production on a large scale began. This was probably at least partly because of the fact that these products are very likely to deteriorate rapidly unless an unusual amount of care is taken in their manufacture. Once it was discovered, however, that reasonably stable products could be put on the market, the demand for them increased steadily from year to year.

The standards of the Food and Drug Administration define mayonnaise as follows:

> "*Mayonnaise, Mayonnaise Dressing, Mayonnaise Salad Dressing.* The semisolid emulsion of edible vegetable oil, egg yolk, or whole egg, a vinegar, and/or lemon juice, with one or more of the following: salt, other seasoning commonly used in its preparation, sugar, and/or dextrose. The finished product contains not less than 50% of edible vegetable oil." [Until 1933, 78% of edible vegetable oil was required.]

No definitions for other salad dressings have been formulated, but it is generally understood that the unqualified term "salad dressing" means a product similar to mayonnaise but containing less egg and oil and usually of a thinner consistency. Various vegetable gums, gelatin, or starch are used to stabilize the emulsion. "French dressing" is an even more variable product than the salad dressings, but it usually consists of an unstable mixture of vinegar and vegetable oil with salt, sugar, and spices; it is frequently thickened by pectin, tragacanth, or other vegetable gums.

The manufacture of mayonnaise and other emulsion-type salad dressings on a commercial scale requires very careful supervision if a relatively stable and superior product is to be obtained. An emulsion must be prepared which will not "crack" even when subjected to considerable temperature variation and to the agitation incident to delivery to the grocer's shelves. Since these emulsions cannot be successfully sterilized, very pure materials must be used and strictly sanitary conditions must prevail during the manufacturing and packaging processes, or decomposition due to bacterial and mold action will occur rapidly. Vacuum packing cannot be used, because a considerable quantity of air is desirable in the finished product to give it the right consistency; thus there is the danger of the oil turning rancid, although this is not likely to happen if all the oil has been perfectly emulsified.

Most manufacturers use frozen or dried egg yolk, which produces a very satisfactory product provided the yolk is carefully handled so that bacterial decomposition does not occur. The egg yolk plays the very vital part of the emulsifying agent, the vitellin surrounding the individual oil droplets and preventing coalescence. It also gives the final product the desired color. Highly purified oils are used and care must be taken to prevent incipient rancidity in the storage tanks. Cottonseed or corn oils are most frequently used in commercial processes; olive oil is more expensive and the product does not keep as well. The vinegar, in addition to acting as a preservative, contributes to the flavor of the product. A high-grade cider vinegar is usually considered to produce the best flavor.

Each manufacturer has his own method of producing the emulsion, and many factors must be considered, such as the temperature, the quality of the ingredients, the proportions used in preparing the primary emulsion, the rate of addition of the oil, and the speed of mixing. The usual procedure is to mix the egg, salt, spices, and part of the vinegar, then add the oil slowly with constant stirring, and finally incorporate the remainder of the vinegar and any water.

Methods of analysis and a formula for calculating the composition for mayonnaise and salad dressings are given in *Methods of Analysis*, of the A. O. A. C., 5th ed., 1940.[22]

---

[22] *Methods of Analysis.* Assoc. Official Agr. Chemists, Washington, D. C., 1940.

Selected Bibliography

Fiene, F., and Blumenthal, S., *Handbook of Food Manufacture.* Chemical Pub. Co., New York, 1938.
Jacobs, M. B., *Chemical Analysis of Foods and Food Products.* Van Nostrand, New York, 1938.
Leach, A. E., *Food Inspection and Analysis.* Revised by Winton. Wiley, New York, 1920.
Schneider, A., *Microanalysis of Powdered Vegetable Drugs.* Blakiston, Philadelphia, 1921.
Winton, A. L., and Winton, K. G. B., *Microscopy of Vegetable Foods.* Wiley, New York, 1916.
Winton, A. L., and Winton, K. G. B., *Structure and Composition of Foods.* Vol. IV, Wiley, New York, 1939.
Woodman, A. G., *Food Analysis.* McGraw-Hill, New York, 1941.
Youngken, H. W. *Textbook of Pharmacognosy.* Blakiston, Philadelphia, 1943.

TABLE 218

CIDER VINEGAR ANALYSES[a]

Determination	Min.	Max.	Av.
Specific gravity (15.6° C.)	1.0126	1.0275	1.0177
Acidity as acetic acid, g. per 100 ml.	3.24	9.96	5.21
Nonvolatile acid as lactic, g. per 100 ml.	0.05	0.30	0.18
Alcohol by volume, g. per 100 ml.	0.03	2.00	0.35
Glycerol, g. per 100 ml.	0.23	0.46	0.30
Total solids, g. per 100 ml.	1.20	4.45	2.40
Reducing sugars as invert sugar, g. per 100 ml.	0.11	1.12	0.47
Volatile reducing substances as invert sugar, g. per 100 ml.	0.14	0.34	0.20
Nonsugar solids, g. per 100 ml.	1.00	2.90	1.90
Pentosans, g. per 100 ml.	0.08	0.22	0.16
Formic acid, g. per 100 ml.	0.0003	0.009	0.003
Volatile esters as ethyl acetate, g. per 100 ml.	0.30	0.91	....
Total ash, g. per 100 ml.	0.20	0.57	0.38
Soluble ash, g. per 100 ml.	0.17	0.51	0.34
Phosphoric acid in soluble ash, g. per 100 ml.	0.007	0.040	0.017
Phosphoric acid in insoluble ash, g. per 100 ml.	0.004	0.032	0.012
Alkalinity of soluble ash, ml. 0.1 *N* acid	21.5	56.0	35.0
Polarization in 200-mm. tube, ° V.	−0.2	−3.6	−1.4
Sugars in total solids, %	5.3	43.3	20.2
Total ash in nonsugar solids, %	12.0	30.0	19.0

[a] R. O. Brooks, *Critical Studies in the Legal Chemistry of Foods.* Chemical Catalog Co., New York, 1927, p. 84. A. G. Woodman, *Food Analysis.* McGraw-Hill, New York, 1941, p. 440.

Most of the adulterations of vinegar consist of the addition of other types of vinegar to cider vinegar or a complete substitution of some other type for cider vinegar. By a careful consideration of all the analytical constants, these can usually be detected rather easily. Some of the constants of cider vinegar may be matched in the adulterated sample, but it rarely happens that all of them can be matched.

IV. MAYONNAISE AND SALAD DRESSINGS

Although mayonnaise and similar types of salad dressings have been made and used in the home in this country for many years, it has been only recently that commercial production on a large scale began. This was probably at least partly because of the fact that these products are very likely to deteriorate rapidly unless an unusual amount of care is taken in their manufacture. Once it was discovered, however, that reasonably stable products could be put on the market, the demand for them increased steadily from year to year.

The standards of the Food and Drug Administration define mayonnaise as follows:

> "*Mayonnaise, Mayonnaise Dressing, Mayonnaise Salad Dressing.* The semisolid emulsion of edible vegetable oil, egg yolk, or whole egg, a vinegar, and/or lemon juice, with one or more of the following: salt, other seasoning commonly used in its preparation, sugar, and/or dextrose. The finished product contains not less than 50% of edible vegetable oil." [Until 1933, 78% of edible vegetable oil was required.]

No definitions for other salad dressings have been formulated, but it is generally understood that the unqualified term "salad dressing" means a product similar to mayonnaise but containing less egg and oil and usually of a thinner consistency. Various vegetable gums, gelatin, or starch are used to stabilize the emulsion. "French dressing" is an even more variable product than the salad dressings, but it usually consists of an unstable mixture of vinegar and vegetable oil with salt, sugar, and spices; it is frequently thickened by pectin, tragacanth, or other vegetable gums.

The manufacture of mayonnaise and other emulsion-type salad dressings on a commercial scale requires very careful supervision if a relatively stable and superior product is to be obtained. An emulsion must be prepared which will not "crack" even when subjected to considerable temperature variation and to the agitation incident to delivery to the grocer's shelves. Since these emulsions cannot be successfully sterilized, very pure materials must be used and strictly sanitary conditions must prevail during the manufacturing and packaging processes, or decomposition due to bacterial and mold action will occur rapidly. Vacuum packing cannot be used, because a considerable quantity of air is desirable in the finished product to give it the right consistency; thus there is the danger of the oil turning rancid, although this is not likely to happen if all the oil has been perfectly emulsified.

Most manufacturers use frozen or dried egg yolk, which produces a very satisfactory product provided the yolk is carefully handled so that bacterial decomposition does not occur. The egg yolk plays the very vital part of the emulsifying agent, the vitellin surrounding the individual oil droplets and preventing coalescence. It also gives the final product the desired color. Highly purified oils are used and care must be taken to prevent incipient rancidity in the storage tanks. Cottonseed or corn oils are most frequently used in commercial processes; olive oil is more expensive and the product does not keep as well. The vinegar, in addition to acting as a preservative, contributes to the flavor of the product. A high-grade cider vinegar is usually considered to produce the best flavor.

Each manufacturer has his own method of producing the emulsion, and many factors must be considered, such as the temperature, the quality of the ingredients, the proportions used in preparing the primary emulsion, the rate of addition of the oil, and the speed of mixing. The usual procedure is to mix the egg, salt, spices, and part of the vinegar, then add the oil slowly with constant stirring, and finally incorporate the remainder of the vinegar and any water.

Methods of analysis and a formula for calculating the composition for mayonnaise and salad dressings are given in *Methods of Analysis*, of the A. O. A. C., 5th ed., 1940.[22]

---

[22] *Methods of Analysis*. Assoc. Official Agr. Chemists, Washington, D. C., 1940.

Selected Bibliography

Fiene, F., and Blumenthal, S., *Handbook of Food Manufacture*. Chemical Pub. Co., New York, 1938.

Jacobs, M. B., *Chemical Analysis of Foods and Food Products*. Van Nostrand, New York, 1938.

Leach, A. E., *Food Inspection and Analysis*. Revised by Winton. Wiley, New York, 1920.

Schneider, A., *Microanalysis of Powdered Vegetable Drugs*. Blakiston, Philadelphia, 1921.

Winton, A. L., and Winton, K. G. B., *Microscopy of Vegetable Foods*. Wiley, New York, 1916.

Winton, A. L., and Winton, K. G. B., *Structure and Composition of Foods*. Vol. IV, Wiley, New York, 1939.

Woodman, A. G., *Food Analysis*. McGraw-Hill, New York, 1941.

Youngken, H. W. *Textbook of Pharmacognosy*. Blakiston, Philadelphia, 1943.

SUBJECT INDEX

A

Absorption by the body, 346–353
  of individual substances, 348–353
  influence of endocrines, 353
  major factors, 346–347
  scheme of, 346
  in small intestine, 346–347
Acacia. See *Gums.*
Acacia species, 821
Acetaldehyde, in metabolism, 108, 143, 204, 806, 807, 808, 809
  in fruits, 774, 776
Acetaldehyde reductase, 204, 218, 223, 226
Acetic acid, 26, 27, 28, 30, 51, 52, 84, 90, 143, 173, 698, 712, 809
Acetoacetate, 107
Acetoacetic acid, 106, 143
Acetoin, 219
Acetone, 84, 143
Acetone bodies, 142
Acetone glycerol, 96
Acetonuria, 106
Acetylcholine, 269
Acetyl number, 97
Acetylphosphate, 219
Achromobacter perolans, 544
Achroödextrin, 78
α-Achroödextrin, 329
β-Achroödextrin, 329
Acid albuminate, 113
Acid-base balance, 282–283, 295–296
  -base equilibria, 270
Acid metaprotein, 113
Acid number, 94
Acidophilus milk, 700
Acidosis, dioic-acid, 107
Acids. See also under specific acid.
  aliphatic, monocarboxylic, 88, 89
  amides of, 102
  amino. See *Amino acids.*
  diamino, 112, 120, 121, 123
  dicarboxylic amino, 120, 124
  fatty, saturated, 96
  and food spoilage, 357, 368
  in large intestine, 354
  organic, in pickling, 377
  polyhydroxy, 79
  unsaturated, 96, 97, 101
Acrinyl isothiocyanate, 888
Acrolein, 98
Actinia, 175
Actiniohematin, 175
Activators. See ***Enzymes, activation of.***
α-Acyl-γ-tritylglyceride, 96
Adenine, 167, 168, 172, 221, 255
Adenosinetriphosphate, 801
Adenylic acid, 169, 204, 221, 238, 255, 800, 801
Adenylpyrophosphates, 801, 802
Adermin, 239
Adrenaline, 143, 144, 262, 267, 468. See also *Epinephrine.*
Adrenosterone, 262
Adsorption, 24, 38–39
  selective, 24, 39
Aerators, in ready-mixed flours, 661
Aerobacillus, 87
Affinity constant, 202
Agar-agar, 36, 40, 51, 819, 822–823
Agaricus campestris, 758
Agmatine, 143, 146
Agnosterol, 99
Alanine, 117, 119, 124, 130, 132, 133, 134, 142, 143, 148, 154, 156, 180, 527, 618, 743, 747
  deamination of, 808, 809
β-Alanine, 183, 242
d-Alanine, 138, 146
Alanyldiglycine, 343
Alanylglycine, 136, 147
Albumen, 22, 37, 39, 547–548
Albumen index, 547–549
Albumin, 46, 111, 113, 117, 128, 209, 464, 617, 618, 619, 620, 647
  blood, 472
  egg, 33, 111, 113, 114, 117, 126, 150, 158, 284, 484–485
  in soybeans, 745
Albuminoids, 112
Alcaptonuria, 143
Alcohol dehydrogenase, 218
Alcohols. See also under name of alcohol.
  polyhydroxy, 78, 79
  trihydric, 97
Aldehyde mutase, 218
Aldehydes, 77
Aldehydrol formula, 60
Aldohexoses, 49, 55, 56, 59, 68, 80
  D^+-galactose series, 55–56
  D^+-glucose series, 56
  identification of possible configurations ,55–61
  structure, 55–61
Aldol, 108
  condensation, 76
Aldonic acids, 78, 79, 80
Aldonolactone, 78
Aldopentoses, 68
Aldoses, 78, 79, 80, 81, 82
Aldotetrose, 49, 65, 67
Aldotrioses, 49, 67
Alewives, 477, 483, 510, 513

Alfalfa, phosphorus content, 279
Algae, fucosterol in, 99
Alimentary canal, 327, 328
Alimentary pastes, 606, 673–677. See also under *Macaroni Products.*
Alizarin, 319, 323
Alkali albuminate, 113
Alkali disease, 292
Alkali metaprotein, 113
Alkaloids, 381, 845
Alkanet, 319
Alkannin, 319
Allergy, 151, 353
Allium
  cepa, 728
  sativum, 729
Allomucic acid, 56
D$^+$-Allose, 56, 70
L$^-$-Allose, 56, 70
Alloxazine, nucleotide, 220
  protein, 216
  protein enzymes, 224
Allspice, 880
Allyl disulfide, 729
Allyl isothiocyanate, 887, 888
Allylpropyl disulfide, 728, 729
Almond, 51, 104, 111, 759
  carbohydrate content, 783
  composition, 778, 812
  oil, 759
  paste, 759
Altrose, 56, 58, 70
Alum, *p*H of, 28
Aluminum, 270, 275, 276, 291, 301
  in cereals, 626
  in fish, 485
  in milk, 407
Amandin, 111, 759
Amaranth, 303, 305
  reactions of, 305
  spot tests, 305
Amebic dysentery, 391
Aminoacetic acid, 119
d-Amino acid oxidase, 170, 171, 217, 223, 226
Amino acids, 110–184, 219, 341, 348, 352, 358, 360
  aliphatic, 119, 121
  analytical procedures, 151–157
  aromatic, 120, 121
  basic, 124, 125, 128, 153, 744
  classification of naturally occurring, 119–123
  color reactions, 152, 153
  complete, 464
  in detoxication, 141–142
  distribution in peptide chains, 163–164
  essential, 178, 180, 256–258, 484, 621, 680
  in flesh of hens, 527
  glycogenic, 142, 796
  heterocyclic, 120
  intermediate metabolism of, use of heavy isotopes, 181–182
    products of, 142–143
  isolation of, 123–128, 182
  ketogenic, 142
  of legumelin, 747
Amino acids (*contd.*)
  of legumin, 747
  nonessential, 180
  in nutrition, 177–182
  optically active, 134
  of pea proteins, 747
  of phaseolin, 743
  as plant constituents, 122
  produced by intestinal putrefaction, 143–146
  properties of, 139–141
  in proteins, 117, 126–127, 618–619. See also individual proteins in this category and throughout index.
  racemic, resolution of, 133–134
  racemization of, 118
  sulfur-containing, 120, 121, 283
  synthesis of, 128–134, 182
  of vicilin, 747
α-Amino-ε-aminocaproic acid, 120
p-Aminobenzoic acid, 230, 243
α-Aminobenzoylpyruvic acid, 143
Aminobutyric acid, 123
α-Amino-*n*-caproic acid, 121
Aminodicarboxylic acids, 147
α-Amino-β-3,4-dihydroxyphenylpropionic acid, 122
β-Aminoethanol, 102
Aminoethyl mercaptan, 144
Aminofumaric acid, 143
α-Aminoglutaric acid, 120
α-Amino-γ-guanidinoxy-*n*-butyric acid, 122
α-Amino-δ-guanidinovaleric acid, 120
α-Amino-β-hydroxy-*n*-butyric acid, 120, 179
α-Amino-β-*p*-hydroxy-3,5-dibromophenylpropionic acid, 122
α-Amino-β-*p*-hydroxy-3,5-diiodophenylpropionic acid, 121
α-Amino-β-hydroxyglutaric acid, 121
α-Amino-β-*p*-hydroxyphenylpropionic acid, 120
α-Amino-β-hydroxypropionic acid, 118
α-Amino-β-imidazolepropionic acid, 120
α-Amino-β-3-indolepropionic acid, 121
α-Aminoisocaproic acid, 120
α-Aminoisovaleric acid, 120
α-Amino-β-ketobutyric acid, 143
Aminolipids, 89
α-Amino-β-methyl-β-ethylpropionic acid, 120
α-Amino-γ-methyl-*n*-butyric acid, 120
Aminopeptidases, 212
α-Amino-β-phenylpropionic acid, 120
Aminopolypeptidase, 343, 795
α-Aminopropionic acid, 119
Aminosuccinic acid, 120
α-Amino-2-thiol-β-imidazolepropionic acid, 122
α-Amino-β-thiopropionic acid, 121
Ammonia, 28, 117, 527, 743, 745, 747, 801
  in proteins, 117
Ammonium compounds, in baking, 652, 687, 691, 701
  carbonate, in mushrooms, 758
  hydroxide, 26
Amyl acetate, in banana, 777
  in synthetic flavors, 898
Amylase(s), 198, 200, 204, 214, 242, 339, 621, 714, 758, 790, 795. See also *Diastase.*

Amylase(s) (*contd.*)
  activity and baking quality, 647–648, 654
  in durum flour, 671
  malt, 194
  salivary, 329, 330, 339
α-Amylase, 622, 648, 671
β-Amylase, 622
Amylograph, 648
Amylopsin, 339, 790, 796
Anacardium occidentalis, 762
Ananas sativas, 780
Anaphylaxis, 151
Anchusa tinctoria, 319
Andropogoneae, 592
Andropogon sorghum, 592
Androsterone, 262
Anemia, 284
  anti-pernicious factor, 255
  and iron, 285–286
Anethole, 881, 886
Anethum graveolens, 885
Aneurin. See *Thiamine.*
Angelica, 300
Angioxyl, 269
Anhydrogalacturonic acid, 820
Anhydro-vitamin A, 234
Anise, 880–881
Aniseed, 880–881
Annato, 319
Anoplopoma fimbria, 489
Anthocyanin, 748
Anthracene, 142
Antibiotic substances, 370, 373
Antibodies, 150–151, 378
Anticatalase, 208
Anti-enzymes, 207, 208
Antigen-antibody, 208
Antigens, 150–151, 177
Antimony poisoning, 381
Antioxidants, 94, 251, 374, 378, 566, 568–569, 578, 579
  in sesame oil, 584
Antipepsin, 208
Antitoxins, 383
Aphanicin, 230, 232
Aphanin, 230, 231
Apiol, 890
Apis
  dorsata, 818
  mellifica, 818
Apium
  graveolens, 738, 882
  petroselinum, 739
Apoenzyme, 216
Apple, 28, 774–776
  carbohydrate content, 783
  cider, 47
  composition, 244, 774, 775, 778, 812
    in storage, 775
  flavor, synthetic, 898
  juice, 27, 29
  raw, 104
  ripening of, 775
Applesauce, composition, 778
Apricot, 769
  composition, 778
  dried, 28
    composition, 778
Araban, 68
  in pecan, 760
Arabinosan, 51, 68
Arabinose, 58, 65, 350
  in gum arabic, 822
  in hemicellulose, 786
  in tragacanth, 822
D^--Arabinose, 61, 65, 68, 70, 77
L^+-Arabinose, 51, 56, 58, 70
Arabitol, 65
Arachidic acid, 90, 745, 760
Arachidonic acid, 91, 256, 464, 565
Arachin, 111, 619, 681
Arachis hypogaea, 760
Arginase, 154, 204, 210, 216
Arginine, 112, 117, 118, 120, 123, 125, 126, 127, 128, 131, 132, 133, 135, 141, 142, 143, 144, 146, 149, 152, 154, 158, 164, 165, 175, 180, 255, 257, 258, 340, 352, 354, 484, 527, 618, 621, 645, 742, 743, 744, 745, 746, 803
Armoracia lapathifolia, 887
Arsenic, 270, 271, 292, 381
  in cereals, 626
  in coloring matters, 318
  in fish, 485–486
Artemisia dracunculus, 893
Artichoke, 731, 757
  French, carbohydrate content, 783
  Jerusalem, 51, 68, 756, 757, 787
Ascorbic acid. See *Vitamin C.*
Ascorbic acid oxidase, 222
Ascorbigen, 244
Ash. See also *Minerals.*
  in bakery products, 710
  in breakfast foods, 678–679
  in cereal grains and products, 629, 630, 631, 632, 635, 641, 655
  in coffee, 844
  in fish, 482
  in various flours, 682
  in foods and feeds, 272, 631, 632
  in fowl, 525
  in fruits, 766, 770, 772, 775
  in the human body, 273
  in meat, 445, 446
  in milk and milk products, 404, 405, 407, 409, 412, 425, 428, 431, 433
  in mushrooms, 757
  in nuts, 778, 779
  in sirups and sugars, 818
  in vegetables, 635, 748, 753, 757–758
Asparagine, 751
Asparagus, 28, 751
  astringency, 757
  blanched-type, 751
  canned, composition, 757
  carbohydrate content, 783
  composition, 751, 757
Asparagus officinalis, 751

Aspartic acid, 117, 120, 124, 125, 128, 131, 132, 133, 142, 143, 154, 164, 180, 567, 618, 743, 747
Aspergillus, 198, 719, 720
Astacin, 492
Astragalus, 822
Atheresthes stomias, 488
Autointoxication, 354
Autolysis, 361
  of fish, 492
  of meat, 447
Auxochrome, 302
Avena species, 592
Aveneae, 592
Avenin, 620
Avidin, 225
Avocado, 776
  carbohydrate content, 783
  composition, 778
Axerophthol, 233
Azlactone method, 130
Azolitmin, 319, 323

B

Babassu oil, 570, 582, 585
  composition, 582
Bacillus
  liodermos, 719
  mesentericus, 713, 714, 715. See also *Rope infection.*
  prodigiosus, 719
  proteus, 390, 543
  pyocyaneus, 543
  subtilis, 371, 542, 719
Bacon, 452, 461
  asphalt-coated, 461
  consumption, 440
  smoked, 104
Bacteria, 34, 110, 134, 173, 204, 254, 284, 349, 354, 355, 357. See also *Food spoilage* and under individual organisms.
  acid-tolerant, 358
  in bakery products, 712–719
  in baking of sour rye bread, 698–700
  in cereal grains, 613
  cryophilic, 363, 364, 365
  in eggs, 542–544
  in fish spoilage, 493
  and food poisoning, 381–390
  in ham souring, 460
  in meat curing, 455, 456, 460
  mesophilic, 363
  rate of growth, 366
  slime-producing, 719
  thermal death time, 363
  thermophilic, 363, 364, 365, 367, 376
Bagasse, 74
Baking and bakery products, 3, 34, 52, 686. See also *Bread*, *Cake*, etc.
  cakes and cookies, 700–706
  composition, 710
  dough. See *Dough.*
  ingredients, 681–687, 688, 689
  laboratory control, 688
  microbiology, 712–723
Baking and Bakery products (*contd.*)
  nutritive properties, 709–712
    caloric value, 710
  packaging, 726–727
  progress of baking industry, 686
  quality control, 718
    and amylase activity, 648
  rope. See *Rope.*
  scoring, 723–726
  sour rye bread, 698–700
  staleness, 706, 709
  wrapping, 708, 720, 721, 726
  yeast-raised products, 688–698, 712. See also *Dough.*
    comparison of formulas, 688–689
    sponge and dough method, 688
    straight dough method, 688
    sweet, 689
Baking powders, 687, 701, 706
Baking soda, 27, 660
Banana, 104, 777
  carbohydrate content, 783
  composition, 778
    and ripening, 777
  dried, composition, 778
  flavoring, synthetic, 898
Barium, 485
Barley, 112, 179, 591, 593, 607–609, 627
  amino acids in, 618
  black, 607, 608, 609
  botanical classification, 592
  bran, composition, 631
  carbohydrate content, 783
  composition, 615, 631
  feed, composition, 631
  flour, composition, 629
  α-glutelin, 618
  hordein, 618
  malt, composition, 631
  malted, 691, 695
  malting, 607, 608, 617
  mineral constituents, 626, 627, 630, 631
  mixed, 608
  pearled, carbohydrate content, 783
    composition, 629
    mineral content, 630
    vitamin B content, 630
  production, 593, 594
  protein content, factors affecting, 616–617
  respiration, 614
  screenings, composition, 631
  six-rowed, 592, 593, 617
  sprouted, 75
  standards, U. S. grain, 607–609
  two-rowed, 592, 593
  utilization, 627, 628
  vitamins in, 624, 625, 630
  western, 607, 608, 609
Barracuda, 479, 489
Bass, black, 489
  sea, 475, 476, 500
  striped, 477
  white, 489
Bassorin, 819

Batyl alcohol, 487
Baudouin test, 576
Bayberry tallow, 90
Bean(s), 28, 111, 251, 300
  adsuki, amino acids in, 619
    globulins in, 619
  carbohydrate content, 783
  carob, 823. See *Locust.*
  dehydrogenases in, 743
  kidney, red, 284, 742
    white, 179, 742
  Lima, 744
    albumin in, 619
    amino acids in, 619, 744
    composition, 281, 757
    dried, 104
    Fordhook, 744
    globulins in, 619
  locust, 819, 823
  mung, amino acids in, 619
    globulins in, 619
  navy, 743
    amino acids in, 619
    comphaseolin in, 619
    composition of carbohydrate, 743
    dry, composition, 757
    minerals in, 279, 286, 288
    phaseolin in, 619
  proteins in, 743
  shell, 742
  snap, 742, 743
    composition, 757
  soy. See *Soybean.*
  string, 742
  tonka, 896
  velvet, amino acids in, 619
    stizolobin in, 619
Beef, 362, 439, 442
  class designation, 442
  composition, 284, 445
  consumption, 439, 440
  corned, cooked, 104
  dark cutting, 449–450
  dried, carbohydrate content, 783
  extract, 169
  fat used for margarine, 588
  grade designation, 442–443
    Institute, 442–443
    U. S., 442–443
  heart, carbohydrate content, 783
  liver, 104, 286
    carbohydrate content, 783
  round, 484, 485
  salted, carbohydrate content, 783
  smoked, carbohydrate content, 783
  tallow. See *Tallow.*
  tapeworm in, 391
Beefsteak, round, 104
Beer, 28, 51, 52
Beeswax, 90, 98
Beet, 28, 748
  canned, composition, 757
  composition, 286, 757
  cooked, carbohydrate content, 783
Beet (contd.)
  fresh, carbohydrate content, 783
  sugar, 748
Beet sugar, 6, 748
Behenic acid, 90, 565
Behen oil, 90
Bence-Jones protein, 117
Benzedrine, 267
Benzene, 142
Benzoic acid, in cranberry, 765
  in synthetic flavors, 897
Berry. See *Fruits.*
Bertholetia nobilis, 761
Beryllium, 270
Betaine, 172, 259
Betanin, 748
Beta
  vulgaris, 748
  vulgaris var. *cicla*, 742
Biebrich scarlet, 303
Bilberry, 318
Bile, 339, 344–346, 350
  composition, 345
  function, 345
  pigments, 345, 354
  salts, 345, 351, 468
  secretion, 344
Bilirubin, 285
Bimolecular reactions, 192, 193
Binders, in food manufacture, 819
Biocatalysts, 224
Biocolloid, 10
Biose, 49
Biotin, 187, 225, 230, 252
  in soybean flour, 682
Biscuit, 659, 660, 661
  mixes, 52
Bismarck brown, 303
  R, 303
Biuret reaction, 152
Bixa orellana, 319
Bixin, 319
Blackberries, 28, 764
  canned, composition, 778
  composition, 778
Blackhead, in turkeys, 524
Blanching, 362, 374
  of Lima beans, 744
  of peas, 746
  of shrimp, 519
Bleaching flours, 696, 702
Bleeding, of gels, 40
Blood, 51, 105, 111, 177, 225, 472. See also under individual blood constituents.
  plasma, 27, 28
  red corpuscles, betaine in, 122
Blood meal, 469
Bloom, in meat, 449
  in poultry, 537
Blue, brilliant, 303, 316, 317
  methylene, 204, 303
  Prussian, 300
  ultramarine, 300

Blueberries, 765
  canned, composition, 778
  composition, 288, 778
Bluefish, 476, 478
Boemer test, 576
Bone, 112, 173, 198
  marrow, 100
Bone black, 39
Bone meal, 469
Borax, *p*H of, 28
Boric acid, 27, 28, 325
Borneol, 892
Borneo tallow, 835
Boron, 294, 485, 626
Botulism, 465
  laboratory diagnosis of, 384
  toxin, 361
Brain, 51, 169
  beef, as food, 465
Bran, 51, 68, 599, 600, 602, 616, 621
  barley, composition, 629
  corn, composition, 631, 636
Bran flakes, composition, 629
  rice, composition, 632, 641, 642, 644, 645
  rye, 648
  wheat. See *Wheat.*
Brassica
  alba, 888
  campestris, 749
  hirta, 888
  juncea, 887
  nigra, 887
  oleracea varieties, 731, 732, 737, 739
  pe-tsai, 739
  rapa, 751
Brazier's method, 125
Brazil nut, 111, 178, 761
  carbohydrate content, 783
  composition of, 778
  oil, 761
Brazilin, 318, 320
Brazilwood, 301, 318, 320
Bread, 3, 4, 27, 28, 34, 604, 653, 688. See also *Flour.*
  basic ingredients, 689
    white, 689
    whole wheat, 689
  bleeding, 719
  bloody, 719
  Boston brown, carbohydrate content, 783
  composition, 710
  doughs for, 52, 688
  enriched, 297, 298, 711. See also *Fortification.*
  and flour quality, 650–654
  freezing, 706
  gas production tolerance in, 693
  gas retention in, 697
  graham, carbohydrate content, 783
    mineral content, 288
  hermetically sealed, 706
  improvers, 691
  minerals in, 711
  moldy, 720
  packaging, 726
  *p*H and mold growth, 721
Bread (contd.)
  propionates in, 717–718
  ropy, 359, 713–718. See also under *Rope.*
    inhibition of, 716–718
  rye, 104. See also *sour rye* below.
  scoring, 724, 726
  slime-producing bacteria in, 719
  sour rye, 698–700
    *p*H, 698
    production by controlled sours, 699
    production by old method, 698
  soy flour in, 684
  staling, 706–707
    measurement, 707–708
    prevention of, 708
  sweating, 40
  toasted, carbohydrate content, 783
  vitamins in, 711
  water rolls, carbohydrate composition, 783
  white, 127
    average, carbohydrate content, 783
    calcium in, 710
    composition, 710
    enriched, 711, 712
    homemade, carbohydrate content, 783
    nutritive properties, 709, 712
  whole wheat, 298, 712
    carbohydrate content, 783
    composition, 710
  yeast-leavened, 652, 653
Breadmaking, 5, 651, 652, 653
Breakfast cereals. See *Cereal preparations.*
Brevoorta tyrannus, 489
Brilliant blue FCF, 303, 316
  reactions, 316
  spot tests, 317
Brilliant orange G, 302
Brine flotation, of peas, 746
  test for potatoes, 752
Broccoli, 731–732
  carbohydrate content, 783
  composition, 757
Broiler, 523, 525. See also *Chicken.*
Bromate, 696
  inhibiting action on enzymes, 623
Bromelin, 468
Bromine, 270, 293, 485, 626
p-Bromophenylmercapturic acid; 142
Brown, Bismarck, 303
  fast, G, 303
Brussels sprouts, 737
  composition, 757
Buckwheat, 594, 628, 630
  amino acids in, 618
  botanical classification, 592
  composition, 615, 616, 630, 632
  feed, composition, 631
  flour, carbohydrate content, 783
    composition, 629, 631
    mineral content, 630
    vitamin content, 630
  globulin in, 618
  kernels, composition, 631
    structure, 600

- *Buckwheat* (*contd.*)
  - middlings, composition, 631
  - mill products, composition, 630
  - mineral constituents, 627
  - production, 593, 594
  - utilization, 628
  - vitamins in, 624, 625
- Buffalo fish, 480, 510
- Buffer index, 403
  - solutions, 29, 30
  - value, 29
- Bulb, regarded as vegetable, 728–729
  - garlic, 729
  - onion, 728–729
- Bullhead, 478, 489
- Bunge theory, 282
- Butanal, 807
- Butanediol, 53, 84
  - 2,3-, 87
- Butanol, 53, 84
- Butter, 5, 10, 45, 47, 93, 104, 559, 565, 570, 580, 585, 586, 588
  - in cake, 706
  - composition, 396, 422–423, 812
  - fat, 95
  - illipe. See *Tallow, Borneo.*
  - leaky, 40
  - production, 396
  - quality standardization, 423–424
  - specific heat, 400
  - types, 422
- Butterfat, 404, 566, 569, 570, 571, 573, 584, 586
  - characteristics, 582, 583
  - composition, 582
  - fatty acids in, 423
  - production, 395, 410
- Butterfish, 476, 510
- Buttermilk, 104, 430–431, 699
  - carbohydrate content, 783
  - composition, 430
  - condensed, 431
  - cultured, 430, 431
- Butternut, 761
  - carbohydrate content, 783
  - composition, 778
- Butter oil, 424
- Butyl sulfocyanide, 887
- Butyrate, 107
- Butyric acid, 90, 106, 108, 565, 573, 582, 712

C

- Cabbage, 28, 358, 737–738
  - carbohydrate content, 783
  - changes in chemical composition, 737
  - Chinese, 739
  - composition, 284, 288, 758
  - unsaponifiable matter in, 738
- Cacao, 824–835. See also *Cocoa* and *Chocolate.*
  - beans, sources, 825
    - West Africa, composition, 833
  - candy, cacao products in, 813
  - components, 834
  - composition, 830
  - consumption of, 827
- *Cacao* (*contd.*)
  - Criollo, 828
  - curing of, 831–832
  - definition, 828
  - exports of, 826
  - fermentation of, 829–831
  - Forastero, 828–829
    - changes during fermentation, 832
    - composition, 832
  - seeds, 828–829
    - characteristics, 834
    - varieties, 828–829, 834
  - tree, 828
    - cultivation, 829
- Cacao-purple, 831, 834
- Cacao-red, 834
- Cadaverine, 143, 145, 354, 360
- Cadmium, 381
- *Caesalpinia braziliensis*, 318, 320
- Caffeine, 322, 831, 835
  - in cassina, 874
  - in coffee, 843, 844
  - in tea, 871, 872
- Caffeol, 843
- Caffetannic acid, 843, 844
- Cake, 659, 661, 700–706, 709, 710, 712, 761
  - angel food, 701, 704, 705, 706, 709, 710
  - balanced formulas for, 704
  - basic formulas for, 705–706
  - batter, 700, 701, 703
  - chocolate, 703, 704
  - cocoa, 703
  - coffee, 688, 689
    - scoring, 725, 726
  - composition, 710
  - devil's food, 704, 709
  - dough, 688, 700, 701
  - driers, 705
  - flour. See *Flour.*
  - fruit, 704, 710
  - high-sugar, 702, 704, 706
  - keeping quality, 703
  - layer, 704, 705, 706
  - low-sugar, 704, 706
  - methods of mixing, 705
  - moisteners, 705
  - molds and, 720
  - nuts used in, 759–763
  - packaging, 726
  - *p*H, optimum, 703–704
  - pound, 704, 705, 706, 710
  - production, 700
    - general aspects, 700
    - leavening, 700–701
    - methods of mixing, 705
  - sponge, 701, 704, 705, 706, 710
  - staling, 709
  - tenderizers, 705
  - tougheners, 705
- Calciferol. See *Vitamin* D_2.
- Calcium, 270, 276–281, 295, 296, 349, 351, 569
  - in blood, 277
  - in bone, 276–277
  - in bread, 710, 711

Calcium (*contd.*)
  in cereal grains and products, 626, 627, 630, 637, 644, 646, 656
  diffusible, 277
  and enrichment of bread, 711
  and enrichment of flour, 662–667
  feed variations, 279
  in fish, 485, 486
  food variations, 279–280
  in various flours, 622, 682
  metabolism, 250
  in milk, 407
  nondiffusible, 277
  -phosphorus ratio, 710
  in pumpkin, 730
  and rickets, 277–278
  in urinary calculi, 278
  vegetables, 737, 738
Calcium compounds. See also under specific compounds.
  acid phosphate, 693, 697, 704
  in baking, 687, 691
  gluconate, 80
  glutamate, 155
  pantothenate, 242
  paracaseinate, 335
  pectate, 820
  phosphate, 469
  phosphatidate, 737
  propionate, 717, 718
Calculi, urinary, 278
Callinectes sapidus, 486
d-Camphene, 886
Canavanine, 122
Candy, 761, 810–839
  annual sales, 811
  definition, 811, 812, 813
  doctors, 816
  ingredients in, 814–819
    annual consumption of, 813
    cacao, 824
    composition of, 812
    flavors, 835–837
    gelatin, 823–824
    gums, 821–823
    pectin, 819–820
    sugars and sirups, composition, 814–819
  total production, 811
Cane sirup, 817
  composition of, 812
Cane sugar, 25, 34, 68, 812
Cannabin, 178
Canned goods, fish, 504–509
  "stacker burn," 509
  storage changes, 365
Cantaloupe, composition, 779
Capric acid, 90, 565, 573, 582
Caproic acid, 565, 573, 582
n-Caproic acid, 90
Caprylic acid, 565, 573, 582, 762
n-Caprylic acid, 90
Capsaicin, 732, 891
Capsicum
  annuum, 732, 891, 892
Capsicum (*contd.*)
  baccatum, 891
  frutescens, 891
Caramel, 53, 78, 320
Caraway, 881
  seed, 881
δ-Carbaminoornithine, 122, 803
Carbobenzoxy, group, 138
  linkage, 147
Carbobenzyloxy group, 138
Carbohydrase, 196
Carbohydrate foods, 782–809
  cellulose, 786
  dextrins, 788
  D^--fructose, 791
  D^+-galactose, 793–794
  D^+-glucose, 792
  glycogen, 788
  hemicellulose, 786
  hydrolysis of, 795
  inulin, 787
  lactose, 789
  maltose, 790
  mannose, 791
  raffinose, 788–789
  stachyose, 788
  starch, 787
  sucrose, 790
Carbohydrate(s), 1, 9, 10, 26, 39, 40, 48, 49–87, 89, 104, 105, 106, 108, 109, 110, 113, 224, 236, 273, 612, 709
  absorption in body, 349–350
  in cereal breakfast foods, 678–679
  in cereal products, 629
  in cereals, 621
  classification, 67–68
    Rosanoff classification, 69–70
  commercial sources, 73–75
    cellulose utilization, 75
    isolation from natural products, 74–75
    starch, controlled hydrolysis, 73–74
  definition, 49–51
  digestion of, 795
  essential, 256, 258–259
  and fats, 569, 806–809
  and food spoilage, 357–359
    fermentation, 358–359
    production of acid, 357–358
  formulation, 49–51
  in fruits, 777, 778–779
  hexopyranoses, α-*D*- series of, 71–72
  in legumes, 743, 745, 746
  metabolism of, 239, 241, 242
  and microbial decomposition, 361
  nomenclature, 64–67
  in nuts, 759, 760, 762, 778–779
  occurrence, 51–53
  oligosaccharides, 792
  oxidation of, 87
  physical properties, 78–79
  and proteins, 806–809
  reducing sugars, reactions, 79–85
  ring formation, 64

Carbohydrate(s) (*contd.*)
  saccharides, 49–87. See under ***Disaccharide, Monosaccharide, Polysaccharide, Trisaccharide, Tetrasaccharide.***
  in sweet potato, changes of, 750
  syntheses of, 76–78
  uses, 51–53
  in vegetables, 635, 748, 750, 757–758
Carbon, activated, 39
Carbon dioxide, in baking, 692, 701
  in fermentation, 692
  and food spoilage, 372
  in oxidation of lactic acid, 803
  in respiration of various fruits and berries, 763, 764, 766, 767, 770, 771, 773, 774, 776, 780
  in respiration of potatoes, 754, 755, 756
Carbonic acid, *p*H of, 28
Carbonic anhydrase, 22, 217, 334
  theory of HCl secretion, 334, 335
Carboxylase, 199, 207, 219, 223, 224
Carboxypeptidase, 214, 340
Carboxypolypeptidase, 211, 212, 796
Cardamon, 881
Carenol, 233
Carica papaya, 198
Carmine, 301
Carminic acid, 321
Carnauba wax, 90, 98
Carob bean, 213, 823
Carotene, 320–321, 569, 585, 623
  in beans, 743
  in fish, 492
  in vegetables, 736, 737, 748, 752
α-Carotene, 230, 232
β-Carotene, 230, 232, 233
  in fruits, 767, 771
  in spinach, 742
γ-Carotene, 230, 231
K-Carotene, 230, 232
Carotenoid, 351
Carotid gland, 269
Carotidin, 269
Carp, 480, 510
  sperm, 112
Carrot, 28, 748–749
  canned, composition, 757
  carbohydrate content, 783
  composition, 748, 757
Carthamin, 318, 324
Carthamus tinctoria, 318, 324
Carum carvi, 881
Carvacrol, 893
d-Carvone, 881
Carya
  illinoensis, 760
  laciniosa, 759
  ovata, 759
Caryophyllene, 884
Caryophyllus aromaticus, 884
Casaba, composition, 779
Caseianic acid, 123
Casein, 43, 75, 112, 113, 114, 117, 118, 126, 128, 175, 178, 194, 198, 200, 209, 283, 335, 398, 404, 484, 485, 619
Caseinic acid, 123
Cashew nut, 761
  composition, 778
  starch in, 779
Casings, 468
Cassava, 595
Cassia, 882, 883
  Batavia, 883
Cassina, 874
Castanea, 759
Castor oil, 90, 95
Catabolism, of D^+-glucose, 797–806
  of glycogen, 797–806
Catalase, 176, 192, 199, 201, 202, 205, 207, 208, 209, 210, 216, 219, 222, 528, 758
  fat, 194
  unit, 209
Catalyst, function, 194. See also *Enzymes*.
Catechin, 831
  tannin, 831
Catechol, 94, 568
Catfish, 478, 480, 484
Cathepsin, 195, 198, 203, 212
Cattle 280, 282, 284, 289, 292, 441
  breeds, 435
  census, 436
  diseases caused by minerals, 278, 285, 287, 292–293
  mineral deficiency diseases, 277–278, 281
  mineral matter, effect on growth, 271–272
Cauliflower, 732
  canned, composition, 757
  carbohydrate content, 783
  composition, 732, 757
Celery, 738
  carbohydrate content, 783
  composition, 757
  seed, 882
  sugars in, changes, 738
Cellobiase, 85
Cellobiose, 64, 68, 85
  4-β-D^+-glucosyl-D^+-glucose, 62
Cellulase, 85
Cellulose, 37, 51, 53, 63, 68, 79, 85, 86, 87, 354, 600, 616, 621, 786, 787, 792
  in coconut, 762
  in legumes, 745, 746
  structure, 63, 786
  utilization, 75
  wrapping, transparent, 726
Cellulose compounds, acetate, 53, 86
  nitrate, 86
  palmitate, 53
  stearate, 53
  xanthate, 86
Cephalin, 89, 100, 101, 102, 177
Cephalopod, 177
Ceratonia siliqua, 823
Cereal feeds, composition, 631
Cereal grains, 182, 295, 372, 591–680. See also under individual grains.
  acidity increase, 613–614
  classification, botanical, 592
  commercial, 603–612

- *Cereal grains* (*contd.*)
  - composition, 615–627
    - ash, 615, 616, 626
    - carbohydrate, 621
    - enzyme, 622–623
    - fat, 615
    - fatty oils, 622
    - fibers, 615
    - lipids, 621–622
    - mineral constituents, 626–627
    - nitrogen-free extract, 615
    - protein, 615, 617–621
    - vitamin, 623–626
  - deterioration, 613
  - germination and enzymes, 622, 623
  - *Gramineae*, 591, 592
  - grass family, 591
  - heating of, 612, 614
  - kernel structure, 598–602
    - buckwheat, 600
    - corn, 600–601
    - oats, 601–602
    - wheat, 598–600
  - legume family, 591
  - moisture and respiration, 613, 614
  - production, 593
    - barley, 593
    - buckwheat, 594
    - corn, 594
    - oats, 595
    - rice, 595
    - rye, 596
    - sorghums, 597
    - wheat, 597–598
  - respiration, 612–614
  - standards, U. S. grain, 603–612
  - storage properties, 612–614
- Cereal preparations, 677–680. See also under individual grains.
  - corn, 678
  - miscellaneous, 697
  - oat, 678
  - production, 677
  - rice, 678
  - rye, 678
  - wheat, 678–679
- Cereal products, 3
  - composition, 629, 631
  - enrichment of, 662
  - fuel value, 629
- Cerebronic acid, 89, 103
- Cerebrosides, 51, 89, 103
- Cerelose, 816
  - composition, 818
- Cerotic acid, 90
- Ceryl alcohol, 98
- *Cetorhinus maximus*, 489
- Cetyl alcohol, 98, 487
- Chalcone, 254
- Charcoal, 38, 300
- Chard, 742
  - carbohydrate content, 783
  - composition, 757
- Chaulmoogra oil, 90
- Chaulmoogric acid, 90
- Cheese, 5, 34, 40, 52, 295, 360
  - cheddar, carbohydrate content, 783
  - composition, 284, 428
  - consumption, 396
  - cottage, 104, 429
  - cream, 429
  - limburger, 380
  - pasteurized, 429
  - process, 429
  - production, 396
  - Swiss, 104, 279
  - varieties, 428
- Cheilosis, 239
- Chenodesoxycholic acid, 345
- Cherbuliez' method, 214
- Cherry, 28, 769–770
  - Bigarreau, 769
  - carbohydrate content, 783
  - composition, 769, 778
  - Duke, 769
  - flavoring, synthetic, 898
  - gum, 51, 68
  - heart, 769
  - Mazzard, 769
- Chestnut, 759
  - carbohydrate content, 783
  - composition, 778
  - starch in, 779
- Chick, 523
- Chicken, 277, 288, 291, 293, 522, 523, 524–528. See also *Poultry*.
  - amino acids in, 619
  - breeds, 524
  - definitions, 525
  - edible portion, composition, 279, 525, 526
    - protein nitrogen, in, 534–535
  - fat of, 527
    - acidity of, 533
  - market grades, 525, 526
  - muscle protein, 619
  - roast, lipid content, 104
  - weight, distribution of, 526
- Chicory, 787
  - and coffee, 856
  - root, 51, 68
- *Chilomastix mesnili*, 391
- Chinese cabbage, 739
  - composition, 757
- Chinese wax, 98
- Chitin, 174
- Chlorine, 270, 272, 282, 295, 626, 627
  - and bleaching flour, 702
  - in milk, 407
- Chlorocruorin, 175
- Chloroflavin, 239
- Chloroform, in synthetic flavors, 897
- Chlorogenic acid, 844
- *Chlorophora tinctoria*, 322
- Chlorophyll, 175, 176, 281, 298, 321, 756
  - in fish, 492
  - hemin residue, 217
- Chlorophyll *a*, 175, 176, 321
  - in cabbage, 737

Chlorophyll *b*, 175, 321
  in cabbage, 737
Chocolate, 17, 52, 784, 824. See also *Candy*, *Cacao*, and *Cocoa*.
  bitter, composition, 812
  carbohydrate content, 783
  definition, 828
  sweet, composition, 812
  sweet milk, composition, 812
Cholagogues, 345
Cholecystokinin, 344
Cholesterol, 93, 99, 100, 278, 345, 351, 405, 406, 487, 573
Cholic acid, 345
Choline, 89, 100, 101, 102, 105, 144, 256, 259–260
  related compounds, 259–260
  in soybean flour, 682
Chondroitinsulfuric acid, 173, 174
Chondroproteins, 173
Chondrosamine, 173
Chondrus crispus, 819
Chroman, derivatives, 251
Chromium, 485
Chromogen, 302
Chromophore, 302
Chromoproteins, 112, 167, 175–177
Chromosomes, 172
Chrysoidine, 303
Chubs, 480, 510
Chylomicrons, 351
Chyme, 330, 337, 346
Chymopapain, 214
Chymotrypsin, 212, 217, 339, 340, 796
Chymotrypsinogen, 215, 339
Chymyl alcohol, 487
Cichorium endiva, 739
Cider, *p*H of, 28
Cinchol, 99
Cinchona bark, 99, 318
Cinchona succirubra, 318
Cineol, 882
Cinnamic aldehyde, 883
Cinnamomum
  burmani, 883
  cassia, 883
  loureirii, 883
  zeylanicum, 882
Cinnamon, 882–883
Cisco, 510
Citral, 897
Citrate, 30
Citric acid, 30, 52, 84
  in fruits, 766, 767, 768, 769, 771, 774, 776, 778–779, 780, 781
  in synthetic flavors, 897
  in vegetables, 731, 736, 739, 742, 751
Citric dehydrogenase, 199
Citrin, 254–255
Citron, 769
  candied, composition, 778
Citrulline, 122, 180, 803
Citrullus vulgaris, 774
Citrus
  aurantifolia, 768
Citrus (contd.)
  grandis, 767
  limonia, 767, 896
  medica, 769, 896
  nobilis, 767
  sinensis, 766
Clam, 104, 475, 516, 517
  canned, 481
  hard, 476, 477, 484
  razor, 479, 480
Claviceps purpurea, 596
Clostridium
  botulinum, 361, 376, 381–384, 465
  welchii, 713
Clove, 884
  phosphorus content, 279
Clupanodonic acid, 91
Clupeine, 112
Coatings in food manufacture, 819
Cobalt, 270, 293, 294, 626
Cocarboxylase, 171, 220, 221, 223, 224, 236
Coccine. See *New coccine*.
Coccus cacti, 321
Cochineal, 321
  carmine, 301
Cockerel, 523. See also *Chicken*.
Cocoa, 104. See also *Cacao*, *Candy*, *Chocolate*.
  carbohydrate content, 783
  composition, 812
  definition, 828
  products, 813
Cocoa butter, 565, 570, 589, 828
  characteristics, 582
  composition, 581
Cocoa red, 322
Coconut, 762
  composition, 778
  dry, composition, 812
Coconut oil, 90, 93, 95, 565, 566, 570, 571, 573, 576, 585, 589, 761, 828, 888
  characteristics, 582, 583
  composition, 582
Cocos
  butyracea, 828
  nucifera, 762, 828
Cod, 475, 476, 479, 483, 484, 486, 488, 491, 500, 510, 513
  black, 489
  iodine in, 288
Codehydrogenase *I*, 170, 221
Codehydrogenase *II*, 170, 221
Cod-liver oil, 90, 91, 95, 288
Coenzyme, 150, 224, 236
  amino acid oxidase, 170, 171
Coenzyme *I*, 218, 221, 223
Coenzyme *II*, 218, 221, 223, 224
Coenzymes, 170–171, 203, 218, 220, 221, 222, 223, 225, 226
  alloxazine, 203
  and conjugated proteins, 222–223
  function, 219–224
  linked reaction, 218
  pyridine, 203

Coenzymes (*contd.*)
  structure, 219–224
  thiamine, 203
Coffalic acid, 844
Coffea
  arabica, 842, 843, 846, 851, 852
  liberica, 842, 846, 852
  robusta, 842, 846, 852
Coffee, 51, 784, 840–856
  acidity of, 844
  aromatic principles, 845
  botany, 842–843
  brewing, 854
  characteristics, trade, 852–853
  chemical aspects, 843
  and chicory, 856
  composition, 843, 844
  concentrates, 855–856
  consumption, 842
  cooling, 848–849
  cultivation, 846
  grinding, 849–851
  Guatemala, 844
    composition, 844
  Java, 851–852
  marketing, 851–852
    preparation for, 846
  Mocha, 844, 851, 853
    composition, 844
  Padang, 844
    composition, 844
  pharmacology, 845–846
  producing areas, 841
  production, 840–842
  roasting, 846–848
  Santos, 844, 851, 852
    composition, 844
  stoning, 848
  types, 852–853. See also *Guatemala*, *Java*, *Mocha*, *Padang*, *Santos* in this category.
Coffee substitutes, 52, 855–856
Cohune oil, 570, 585
  characteristics, 582, 583
  composition, 582
Colamine, 89, 100, 102
Collagen, 112
Colloid mill, 36, 42
Colloid(s), 2, 22, 35–47
  chemistry of, 35–47
  classes, 35, 36
  emulsions, 41–45. See also *Emulsion.*
  foams, 45–46
  gels, 39–40. See also *Gel.*
  hydrated, 41
  hydrophilic, 39, 41, 42, 44, 652
    water removal from, 39
  hydrophobic, 43, 44
  imbibition, 40–41
  lyophilic, 36, 37, 38, 39, 41
  lyophobic, 36, 37
  preparation, 36
  properties, 36–39
  sol, 39–40. See also *Sol.*
Coloring matters, 300–326. See also under name of individual dye.
  dyes, acid, 301
    basic, 301
    certified, 303
    coal-tar, 301–318
    lakes, 301
    natural, organic, 318–326
  pigments, definition, 300
    in fish, 487, 492
Colza oil. See *Rapeseed oil.*
Comphaseolin, 619
Complement. See *Enzyme, activation of.*
Compressometers, 708
Conalbumin, 619
Conarachin, 619, 681
Condiments, 876, 901
Confectionery. See *Candy.*
Conglutin, 179
Congo red, 274
Coniferin, 895
Consommé, canned, carbohydrate content, 783
Cookies, 700–706, 709
  composition, 710
  flour for, 701–703
  packaging, 726
Copper, 177, 270, 289–290, 464
  in berries, 763, 765, 773
  in cereals, and cereal grains, 626, 630, 644, 734
  in cucurbits, 730, 731
  in fish, 485, 486
  in foods, 289–290
  in fruits, 740, 767, 768, 769, 770, 771, 774, 775, 776, 777, 780
  in legumes, 743, 744, 745
  in milk, 407
  in peanut, 760
  as prooxidant, 568
  in vegetables, 732, 733, 737, 738, 739, 740, 742, 747, 748, 749, 751, 752, 756
Copper sulfate, 300
Copra, 762
Coprasterol, 99
Coral, 122
Coriander, 884
Coriandrol, 884
Coriandrum sativum, 884
Cori ester, 204
Corn, 28, 75, 87, 112, 128, 594, 600–601, 609, 632–637
  acidity, 637
  albumins, 127
  amino acids in, 618
  bran, composition, 631
  breakfast foods, 678. See also *Corn flakes.*
  calcium content, 279
  canned, 757
  classes, 609
  composition, 615, 616, 757
  dent, corn, 592, 594, 600, 615, 616
  distillers' grains, composition, 631
  dry-milled, 635–636
    chemical composition, 636
    definition and standards, 635–636

Corn (*contd.*)
  ear, chops, 636
  feed by-products, 637
  feed meal, composition, 631
  flint, 592, 594, 600, 615, 616
  fodder, phosphorus content, 279
  germ, 127
    meal, composition, 631
  glutelin, 618
  gluten, 127
    feed, composition, 631
    meal, composition, 631, 637
  grits, 635
  hominy. See *Hominy.*
  hybrid, 594
  kafir, 102
  kernel, chemical composition, 633, 635
    structure, 600–601
  maize. See *Maize.*
  mixed, 609, 610
  oil, 565, 571, 576, 579, 584, 589
    cake, 637
    characteristics, 582
    composition, 581
    meal, composition, 631
  popcorn, 592, 600, 615, 616, 726
  production, 593, 594
  respiration, 614
  soundness, index of, 637
  standards, U. S. grain, 610
  sugar. See *Dextrose.*
  sweet, 592, 594, 600, 615, 616, 733–736
    canned, composition, 757
    changes in chemical composition, 734–735
    composition, 757
    fresh, carbohydrate content, 783
  utilization, 632–633, 634
  vitamins in, 624, 625
  wet-milled, definitions and standards, 637
  white, 609, 610, 623, 624, 625, 633
  yellow, 127, 609, 610, 623, 624, 625, 633
  zein, 618
Corn bread, 104
Corncobs, 51, 68, 84
Corn flakes, 104
  composition, 629
Corn flour, 633, 636
  composition, 629, 631
Corn meal, 629, 633, 635, 636
  carbohydrate content, 783
  composition, 629, 631
  mineral constituents, 627
  mineral content, 630
  vitamin content, 630
Corn sirup, 47, 51, 818
  in candy, 813, 814, 815–816
  composition, 812, 818
  preparation, 73–74
Cornstarch, 53
  composition, 812
Corticosterone, 266, 267
Corylus, 759
Cottonseed, 178
  flour, 680–684
Cottonseed (*contd.*)
    caloric value, 682
    composition, 681–683
    mineral constituents, 682
    production, 680
    uses, 683–684
    vitamins in, 682
  globulins, 619
  hulls, 51, 84
  meal, phosphorus content, 279
  oil, 7, 91, 94, 95, 571, 576, 577, 579, 584, 586, 587, 589, 590
    composition, 581
    hydrogenation, 576
    winterized, 589
Coumarin, 896
Coumarouna odorata Aublet, 896
Cowpea, dried, carbohydrate content, 783
  globulin amino acids in, 619
  green, carbohydrate content, 783
Cozymase, 204, 218, 220, 221, 223, 224
Crab, blue, 484, 486
  canned, 481
  hard, 475, 477, 478, 479, 480, 516, 518
  soft 477
Crackers, 28, 688, 689, 703
  basic ingredients, 689
  carbohydrate content, 783
  composition, 710
  dough, 688
  manufacture, 653
  packaging, 726
Cranberry, 765
  carbohydrate content, 783
  composition, 778
  sauce, composition, 778
Cream, 36, 42, 43, 45, 47, 397, 399, 418–422
  carbohydrate content, 783
  churning, 45
  composition, 418–419
  dried, consumption, 396
    production, 396
  fat globules in, 401
  grades, 418–419
  heavy, composition, 812
  homogenized, 43
  plastic, 421
  sour, 420
  specific heat, 401
  viscosity, 45
  whipped, 46, 421
Cream cheese, 429
Cream of tartar, 717
  in cake, 704, 706
Creatine, 260, 352, 801
Cresol, 145
Cretins, 287
Criodictyol, 254
Croaker, 477, 478, 483, 484, 500
Croceine, orange, 308
  scarlet, 303
  scarlet 8B, 303
  scarlet O, 303
Crocetin, 325, 892

Crocin, 892
Crocus sativus, 325, 892
Crotonaldehyde, 108
Crotonate, 107
Croton oil, 90
Crumb staleness. See *Bread, staling.*
Crustacea, 516–519
Crustaceans, 177
Cryptoxanthene, 230, 231, 232
Cryptoxanthin, 623
Cucumber, 104, 330
  carbohydrate content, 783
  composition, 757
Cucumis
  melo, 773
  sativus, 729
Cucurbita
  maxima, 730
  pepo, 729, 731
Cucurbits, 729–731
  cucumber, 729
  pumpkin, 729–730
  summer squash, 730–731
  winter squash, 731
Culpea pallasii, 489
Cuminal, 885
Cumin seed, 885
Cuminum cyminum L., 885
Curcuma, 885
Curcuma longa, 319
Curcuma longa L., 325, 885
Curcumin, 319, 325, 885
Currant, 318, 764
  carbohydrate content, 783
  composition, 778
  Zante, 765, 772
Cusk, 475, 500, 510
Cyanidin, 318
Cyclol theory, 166
Cyclopentanoperhydrophenanthrene, 98
Cydonia oblonga, 776
Cynara scolymus, 731
Cynoscion nobilis, 489
Cyprinine, 112
Cysteine, 121, 142, 143, 144, 146, 149, 150, 152, 155, 283
Cysteine thioacetal of formaldehyde, 122
Cysteylglycine, 139
Cystine, 117, 118, 121, 123, 126, 127, 128, 131, 132, 133, 134, 139, 142, 143, 144, 149, 150, 152, 153, 154, 155, 158, 164, 175, 179, 180, 182, 272, 283, 352, 484, 618, 621, 645, 681, 743, 744, 745, 747
  in large intestine, 354
l-Cystine, 128
Cytase, 622, 691
Cytochrome, 176, 199, 217, 221
  system, 170
Cytochrome *a*, 217
Cytochrome *b*, 217
Cytochrome *c*, 217, 221, 222
Cytochrome oxidase, 176, 199, 210, 216, 217, 222, 226
Cytochrome peroxidase, 216
Cytochrome *c* peroxidase, 222
Cytochrome *c* reductase, 217, 221, 223
Cytoglobulin, 112
Cytoplasm, 112, 168, 213
Cytosine, 167, 168, 172

D

Dadhi, 432
Dahlia, bulb, 51, 68
Dairy products, 105, 395–434. See also under individual product.
Dakin's extraction, 116, 124
Damsons, composition, 779
Dandelion, greens, carbohydrate content, 783
  roots, 68
Date, 28, 779–780
  carbohydrate content, 783
  composition, 778
Daucus carota, 320, 748
Dehydration, 39, 84, 378
Dehydroascorbic acid, 225, 245
7-Dehydrocampesterol, 246
7-Dehydrocholesterol, 245, 246, 247, 248, 249
Dehydrocorticosterone, 266
Dehydrogenase, 199, 207, 218, 221, 223, 241. See also under specific dehydrogenase.
  in beans, 743
7-Dehydrositosterol, 246
7-Dehydrostigmasterol, 246
Delphinidin, monoethyl ester, 772
Density, 10, 11–12, 574
  definition, 11
  measurement of specific gravity, 11–12
  of oils and fats, 574, 582
    change with temperature, 583
  use of specific gravity, 12
Desoxyaldohexoses, 68
Desoxyaldopentoses, 68
Desoxyaldotetroses, 67
Desoxycholic acid, 345
Desoxycorticosterone, 266, 267
d-2-Desoxyribose, 168
Desserts, frozen, 426–427
Detoxication by amino acids, 141–142
Detritylation, 96
Dewberry, 764
Dextrans, 68
Dextrin, 36, 52, 53, 75, 200, 815, 818
  in candy, 816
Dextrins, 51, 68, 78, 85, 615, 621, 622, 692, 792. See also *Achroödextrin, Erythrodextrin.*
  in coconut, 762
  hydrolysis, 795
  in navy bean, 743
  in soybean, 745
  in sweet potato, 750
Dextrose, 68, 690, 691, 816, 818. See *D*$^{+}$*-glucose.*
  in baking, 691, 692
  in sweet potato, 750
Diabetes mellitus, 268
Diaceturia, 107
α,β-Diacyl-γ-tritylglyceride, 96
Dialysis, 37
  ultrafiltration, 37

Diaminoadipic acid, 123
Diaminoglutaric acid, 123
Diaminomonocarboxylic acid, 120
Di-α-amino-β-thiopropionic acid, 121
Diaminotrihydroxydodecanic acid, 123
Diastase, 75, 85, 329, 359, 361, 691, 692, 693, 713, 714. See also *Amylase(s)*.
  activity, 34
  effect of milk on, 639
  in flour, control of, 933
Dibromotyrosine, 122
Diethylstilbestrol, 264
Digestion, 327–355. See also *Absorption, Bile.*
  of carbohydrates, 795
  gastric, 330–337
  glands of, accessory, 327, 328
  intestinal, 337–346
    function of large intestine, 353–354
    secretions, 341–343
  nature of, 327–328
  peptic, 200, 337
  of proteins, 352–353
  salivary, 329–330. See also *Saliva.*
  of sugars, 795
Diglyceride, 580, 703. See also *Glyceride(s)*.
Dihydrocholesterol, 99
Dihydrocozymase, 211
22-Dihydroergosterol, 246
Dihydroxyacetone, 49, 50, 67, 78
  phosphate, 802, 806
  phosphoric acid, 800
1,3-Dihydroxy-2-aminoöctadecene-4, 103
1,2-Dihydroxy-3-aminoöctadecene-4, 102
Dihydroxydiaminosuberic acid, 123
2,5-Dihydroxyphenylacetic acid, 143
Dihydroxyphenylalanine, 122
2,5-Dihydroxyphenylalanine, 143
2,5-Dihydroxyphenylpyruvic acid, 143
2,3-Dihydroxypropanal, 49, 803
1,3-Dihydroxypropanone, 49
β-[3,5-Diiodo-4-(3′,5′-diiodo-4′-hydroxyphenoxy)-phenyl]-α-aminopropionic, 122
3,5-Diiodothyronine, 149
Diiodotyrosine, 121, 144, 154
3,5-Diiodotyrosine, 131, 132, 145, 148, 149, 268
Diketopiperazines, 130, 131, 134–139, 141
  complexes, 164
  occurrence, 146
  properties, 146–148
  synthesis, 134–139
Dill seed, 885
p-Dimethylaminobenzaldehyde, 152
Dimethyldelphinidin, monoglucoside of. See *Oenin.*
Di-"oleo"-monosaturated glyceride, 92
Dioleo-palmitin, 95
Diose, 49, 67
Dipeptidase, 195, 198, 211, 212, 216, 343
  yeast, 194
Dipeptides, 135, 136, 141, 146, 212, 344
Diphenylacetylornithine, 142
Diphosphoglyceric acid, 218
Diphosphopyridine nucleotide, 170, 220, 221
Diphosphoric esters, 198
Diphosphothiamine, 223
Dipteryx odorata Aubl., 896
Disaccharide, 51, 68, 78, 82, 85–87, 349
  structure, 62, 64
Dispersion medium. See *Colloids, chemistry of.*
Distilled liquors, 52
Djenkolic acid, 122
Djenkol nuts, 122
Dockage, in cereals, 607, 612
Doctor. See *Candy.*
Dodecandiaminodicarboxylic acid, 123
Dogfish, 483, 489
Dog food, canned, 469
  composition, 469
Dolphin oil, 90
Donnan membrane equilibrium, 274–276
Dough, 21, 22, 34, 688
  cake, 700, 701
  conditioners, 687, 688, 689, 691, 696
  elasticity, 695, 696
  pie, 703
  sour, 699
  sponge and, method, 688
  sticky, 695, 696, 697
  straight, method, 688
  yeast-raised, 688–698
    gas production, 689–693
      dextrins in, 692
      diastatic enzymes, 691, 693
      inorganic compounds, effect of, 691
      *p*H, 692, 693
      soluble starch in, 692
      sugars in, 690
      tolerance, 693
      by yeast, 689–690
    gas retention, 694–698
      acids in, 697
      alcohol in, 697
      control by oxidation, 696
      gluten proteins in, 694, 695
      measurement of, 697
      by mechanical action, 694
      milk, effect of, 694, 696
      mineral salts in, 697
      proteases. See *Proteases.*
      shortening in, 697
      tolerance, 698
      water, effect of, 697
Doughnut, 659, 661, 662, 700, 709, 726
  composition, 710
Drum, 478
Duck, 104
Duodenum, *p*H of contents, 28
Durum wheat. See *Wheat, durum.*
Dutch pink, 301
Dye. See *Coloring matters.*

E

Echinenone, 220, 231
Edestan, 113
Edestin, 111, 113, 117, 178, 194, 209
  isolation of, 115
Eel, 489, 510, 513
Egg(s), 22, 34, 173, 182, 295, 522, 540–563
  air cell in, 546, 551

Egg(*s*) (*contd.*)
  albumen. See *Albumen.*
  albumen index, 547–548
  albumin. See *Albumin.*
  amino acids in, 619
  bacteria in, 542–544
  blood in, 547
  in cake, 704, 705, 706, 709
    beaten, as leavening, 701
  calcium in, 279
  candling, 545
  checked, 550
  composition, 540–542, 812
  conalbumin, 619
  dried, 559–562
    bacteria in, 560
    score of, 562
    storage of, 561
  frozen, 553–559
    in baking, 687, 689, 705
    breaking facilities, 554–556
    holding freezer, 554, 557–558
  grades, 549–550
  grading, 545
  hen's, proteins in, 178
  iron in, 286
  leakers, 550
  liver spots in, 547
  in macaroni products, 674
  meat spots in, 547
  moldy, 542, 546
  oiling of, 553
  ovalbumin, 619
  *p*H, 542
  protein. See *Protein.*
  quality, maintenance of, 544
    shell eggs, 549–550
    specifications, 549–552
    terms used, 550–551
    Van Wagenen chart, 548
    white, 552
    yolk, 551
  refrigeration of, 563
  scrambled, 104
  shell, composition, 540
    processed, 552–553
  sour, 543
  spoilage, 546. See also *quality* above.
  transportation of, 562–563
  vitellin, 112, 117, 619
  white, 34, 36, 41, 45, 46, 47, 110, 174, 225, 547, 552
    composition, 279, 284, 541
    foam, 45
    frozen, 559
    viscosity, 547, 552
  yolk, 42, 45, 47, 100, 111, 112, 126, 177, 547–549, 551
    coloring matter, 320
    composition, 541–542
    frozen, 559
Eggplant, 732
  carbohydrate content, 783
  composition, 757
Ehrlich's reaction, 152
Elaidic acid, 97
Elastin, 112
Electrode, glass, in *p*H determination, 32
Electrodialysis, 37, 115
Elements. See also *Minerals* and under individual elements.
  electronegative, 270
  electropositive, 270
  trace, 270, 288–294
Elettaria cardamomum Maton, 881, 882
Embryo. See *Germ.*
Emulsification, 24
Emulsifiers, 24, 42, 43, 47, 52, 703
  in cake, 705
Emulsin, 73, 82
Emulsion, 10, 36, 62. See also *Homogenization.*
  edible, 46–47
Endamoeba histolytica, 391
Endive, 739
  composition, 281, 757
Endocrines, influence on absorption, 353
Endopeptidase, 212, 336, 340
Endoprotease, 213
Endosperm, 599, 601, 602, 622, 626
English walnut. See *Walnut.*
Enolization, 85
Enteritis, 388, 390
Enterogastrone, 332
Enterokinase, 203, 339, 342, 795
Enterotoxin, 361
Entosphenus tridentatus, 489
Enzyme(s), 10, 164, 168, 185–228, 342, 357, 374. See also under specific enzymes.
  activation of, 202–204, 800
    by sulfhydryl compounds, 623
  activity, 25, 34
    as catalysts, 186–213
    inhibition of, 204, 208
  autolytic, 114, 361
  catheptic, 203. See also *Cathepsin.*
  of cereals, 622–623
    inhibitory action of oxidizing agents, 623
    proteolytic, 622, 623
  as chemical compounds, 213–214
  classification, 226–228
  and coenzymes, 219–224
  co-substrate effect, 344
  definition, 185
  desmo-, 213
  diastatic. See *Amylase* and *Diastase.*
  endo-, 213
  in food spoilage, 361–362, 373–374
  gastric, 334–336, 795
  and hormones, 224–226
  hydrolytic, 196, 197, 198, 200, 202, 374
  inhibition of, 202–208, 215
  of intestinal mucosa, 342–343
  intracellular, 213, 342, 344, 352
  kinetics, 188–210
    determination of reaction order, 190–192
    effect of enzyme concentration, 197, 200
    influence of H-ion concentration, 195–197
    influence of substrate concentration, 200–202
    influence of temperature, 193–195

Enzyme(s) (contd.)
    methods, 189, 190
    temperature coefficients, 194
  lipolytic, 335, 360. See also *Lipase.*
  Michaelis constants, 198–199
  in mushrooms, 758
  oxidative, 202, 216, 217, 218. See also *Amino acid oxidase, etc.*
  pancreatic, 194, 338, 339–341, 528, 796
  Pasteur, 216, 222
  *p*H optima, 198–199
  in poultry fat, 527–528
  prosthetic group of. See *Prosthetic group.*
  as proteins, conjugated, 216–219, 222, 223
    simple, 214–216
  proteolytic, 116, 117, 123, 134, 183, 204, 211, 212 213, 215, 216, 335, 336, 343, 362, 468, 622, 623, 687, 714, 795. See also *Peptidase, Protease, Proteinase, etc.*
  respiratory, 216, 217, 221, 222
    in grains, 613
  specificity of, 210–213
  units, 187, 208–209
  and vitamins, 224–226
  yellow. See *Yellow enzyme.*
Eopsetta jordani, 488
Ephedrine, 267
Epi-7-dehydrocholesterol, 246
Epi-ergosterol, 246
Epimeric, change, 56, 66
  acids, 57
  nitriles, 57
  sugars, 58, 61, 67
Epinephrine, 267, 352, 798. See also *Adrenaline.*
Equilenin, 263, 264
Equilin, 263, 264
Erepsin, 343
  pancreatic, 194
Ergonovine, 596
Ergosterol, 99, 100, 245, 246, 247, 248, 249, 596
Ergot, 99, 100, 122, 596
Ergotamine, 596
Ergothionine, 122, 283
Ergotism, 381
Ergotoxine, 596
Eriodictin, 254
Erucic acid, 90, 565
Erythrocruorin, 162
Erythrodextrins, 78, 329
Erythrolitmin, 319
D^--Erythrose, 50, 70
L^+-Erythrose, 70
Erythrosin, 306
Erythrosin B, 303
  reactions, 306
  spot tests, 306
Erythrosin G, 306
L^--Erythrulose, 50
Escherichia, 390
Escherichia coli, 199, 371, 518, 543, 713
Esterase, 211
Estradiol, 264
α-Estradiol, 263
β-Estradiol, 263
Estriol, 263, 264
Estrogen, 263–265
Estrone, 264
Ethyl alcohol, 52, 84, 87
Ethylamine, 143
Ethyl butyrate, 194, 198
  in synthetic flavors, 898
Ethylcellulose, 86
Ethylene ripening. See *Fruit(s), ripening.*
Ethyl mercaptan, 144
Ethyl vanillin, 836, 837
Eugenol, 895
  in spices, 880, 883, 884
Eulamia
  aethalorus, 489
  azureus, 489
  lamiella, 489
  taurus, 489
  velox, 489
Excelsin, 111, 178
Exoenzyme, 374
Exopeptidases, 212, 336
Exoproteases, 213
Exotoxin, 383
Extensimeter, 22

F

Factor U, 255
Fagopyrum escutentum, 592
Farina. See also *Wheat flour.*
  carbohydrate content, 783
  composition, 629
Farinograph, 21, 22, 695, 695
Fast acid fuchsin, 302
Fast brown G, 303
Fast green FCF, 303, 315, 317
  reactions, 315–316
  spot tests, 316
Fast red E, 305
Fast wool yellow, 305, 312
Fat(s), 2, 6, 9, 14, 16, 41, 48, 52, 88, 89, 90, 91, 92, 93, 104, 110, 224, 236, 273, 350, 351, 564–570, 709. See also *Oil(s).*
  acids derived from, 96–97. See also *Fatty acids.*
  adsorption, chromatographic, 574
  animal, 90
  antioxidants, 568
  in bakery products, 704, 710
  in breakfast foods, 678–679
  and carbohydrates, 569, 806–809
    food spoilage, 360
    microbial decomposition of, 361
  in cereal feeds, 631, 632
  in cereal grains and cereal products, 629, 630, 631, 632, 635, 641, 646, 655
  characteristics, 581–583
  chemical behavior, 92–95
  chicken, 527, 533
  composition, 581–582
    determination of, 575
  density, 574, 582
  deterioration, tests for, 577
  distinction between fats and oils, 567
  edible, 564–590

Fat(s) (*contd.*)
  in edible preparations, 470
  examination of, by chemical methods, 572–573
  in various flours, 682
  in fruits, 770, 778–779
  glycerides. See *Glyceride(s)*.
  Hehner value, 573
  hydrogenated, 574, 580, 583, 587
  iodine value, 95, 572, 581, 583
  Kirschner value, 573
  meat, 465–467
    properties for determining quality, 466
  in meat, 445, 446
  melting point, 92, 95, 574, 582, 583
  in milk and dairy products, 43, 45, 46, 401, 404, 405, 406, 409, 410, 411, 412, 419, 420, 421, 422, 423, 425, 426, 428, 430, 431, 433
  mowrah, 92
  in mushrooms, 757
  nature of, 564–570
  nonglyceride components, 566
  non-nutritional functions, 569–570
  in nuts, 778–779
  oils, specific, tests for, 576–577
  by physical methods, 573–574
  plastic, 586
  Polenske value, 573
  poultry, 527, 528
  production, 571
  and proteins, 806–809
  rancid, 380
  refractive index, 574, 581, 582, 583
  Reichert-Meissl value, 573
  role in human nutrition, 569
  saponification value, 95, 572, 581, 582
  smoke point, 580
  solidification point, 95, 574
  sources, 570–571
  specialty, 589–590
  spoilage of, 567–568
  stability tests, 577
    Schaal method, 578
    Swift method, 578
  storage in body, 104
  thiocyanogen value, 572
  titer, 582–583
  unsaponifiable matter, 95
  utilization, 585, 588–589
  in vegetables, 748, 757–758
  wool, 95, 99
Fatty acid(s), 89, 91, 96, 345, 350, 351, 564–566
  derived from lipids, 90–91
  essential, 104, 256, 464
  esters of, 211, 487
  in fats and oils, 565
  free, 573, 584
  of various nut oils, 759, 760, 761, 762
  saturated, 565, 572, 575
    fungistatic properties, 721
  separation of, 572, 573
  tagged, 104
  unsaturated, 565, 572, 575
Fatty alcohols, 487
Favism, 381
Feces, 28, 99, 353
Feeds, animal, 271, 272, 279, 469. See also *Food*, *Gluten*.
Fenchone, 886
Fennel seed, 885
Fermentation, 25, 34, 80, 84, 87, 204, 216, 238, 354, 360, 712, 713, 718
  in baking, 688, 689, 691, 692
    and sour rye bread, 699
    tolerance, 698
  of cacao seeds, 829–831
  of coffee, 846
  of flour, 652, 653
  and food spoilage, 358
  gaseous, 365
  of hexoses, 806
  optimum temperature, 712
  of tea, 863, 865
  of vanilla, 894
Ferment, 213, 222, 698
Ferrocytochrome c, 210, 222 .
Fertilizer, 276, 471. See also *Enzymes*.
FD&C Blue No. 1, 303, 316
  No. 2, 303, 317
FD&C Green, No. 1, 303, 313
  No. 2, 303, 314
  No. 3, 303, 315
FD&C Orange No. 1, 303, 308
  No. 2, 303, 309
FD&C Red, No. 1, 303, 304, 308
  No. 2, 303, 305
  No. 3, 303, 306
  No. 4, 303, 306–307
  No. 32, 303, 307–308
FD&C Yellow No. 1, 303, 309
  No. 2, 303, 310
  No. 3, 303, 310
  No. 4, 303, 311
  No. 5, 303, 312
  No. 6, 303, 313
Fiber, in bakery products, 710
  in breakfast foods, 678–679
  in cereal grains and products, 629, 630, 631, 632
  in various flours, 682
  in fruits, 778–779
  in mushrooms, 757
  in nuts, 778–779
  in vegetables, 741, 742, 743, 748, 757–758
  in wheat and wheat products, 655
Fibrin, 113
Ficin, 214
Ficus carica, 780
Figs, 780
  acetic acid in, 780
  composition, 778
  dried, carbohydrate content, 783
    composition, 812
Filbert, 759
  composition, 778, 812
  starch in, 779
Fillers, in food manufacture, 819
Finnan haddie, 510
Fire point, of fats and oils, 583
Firming agent, for fruits, 52

- Fischer's ester method, 124
- Fischer's synthesis, 138
- Fish, 3, 27, 34, 182, 473–516
  - autolysis, 493
  - by-products, 514–516
    - body oils, 515
    - liver oils, 514–515
    - meal, 515
    - oil, 515
  - canned, 279, 478, 479, 485, 504–509
    - inspection of, 508–509
    - quality of, 508–509
    - salmon, 481, 483, 486, 504–507
    - tuna, 507–508
  - composition, 481–493
    - essential amino acids, 484
    - glycogen in, 492, 493
    - gross, 482–483
    - mineral content, 485–487
    - oil content, 483, 488–489
    - protein content, 483–485
    - variation in, 482
    - vitamin content, 488–492
  - dried, 514
  - fillets, 476
  - fresh, 497–501
  - frozen, 501–504
    - alteration caused by freezing, 501–502
    - alteration caused by storage, 501–502
    - method of freezing, 503
    - protection in cold storage, 503–504
  - handling, in port, 500
    - on vessel, 497–499
  - liver oils, 490–491
  - marketing, 500–501
  - method of freezing, 503
  - oil, 234, 487, 565, 566, 567, 570, 571, 577, 584, 589
    - components of, 487
  - *p*H of, 493
  - pigments in, 487, 492
  - salted, 511–514
    - production, 513
  - smoked, 477, 510–511
    - production, 510
  - spoilage of, 493–494
  - technology, bibliography, 519–521
- Fisheries, 473–481
  - Alaska, 480
  - Chesapeake Bay States, 477
  - Gulf States, 477–478
  - inland lakes and rivers, 480, 481
  - Middle Atlantic States, 476–477
  - New England States, 474–476
  - Pacific Coast States, 478–479
  - South Atlantic States, 477–478
  - world, 473–474
- Fishing methods, 494–497
  - dredges, 475, 476, 477, 478, 517
  - lines, 475, 476, 478, 479, 480, 494
    - trawl, 495
    - troll, 478, 479
  - nets, 476, 477, 479, 495
    - Fyke, 480

Fishing methods (contd.)
    - gill, 476, 478, 480, 496
    - pound, 476, 477, 480
  - otter trawls, 475, 476, 496
  - pots, 475, 476, 479, 480
  - seines, 477, 478, 479, 480
    - haul, 478, 480
    - purse, 476, 477, 496
  - traps, 475, 480
- Fitelson test, 576
- Flagellate diarrhea, 391
- Flagellates, 391
- Flash point, of fats and oils, 583
- Flat sour, 365
- Flavanone, 254
- Flavineadenine dinucleotide, 170, 217, 221, 223
- Flavinmonucleotide, 170
- Flavoproteins, 170, 216, 217, 219, 221, 222, 223
- Flavoring extracts, 893–898
  - definition, 893
  - lemon, 896
  - synthetic, 897–898
  - vanilla, 894
- Flax, respiration of, 614
- Flounder, 475, 476, 479, 483, 486, 488, 491, 500
- Flour, 21, 27, 28, 604, 686, 688, 689, 690, 694. See also *Gluten.*
  - baking quality and enzymes, 623
  - bleaching of, 696
  - blended and prepared, 659–662
  - bread, 696, 702. See also under *Bread.*
  - cake, 701–703, 704. See also under *Cake.*
    - absorption, 702
    - acidity, 702
    - fineness, 702–703
    - granulation, 702
    - *p*H, 702
    - protein, 702
  - carbohydrate content, 783
  - cookie, 701–703
  - cottonseed, 680–684
  - diastatic activity, 693
    - control of, 693
  - enriched, 662–667. See also *Fortification.*
  - hydration capacity, 22
  - improvers, 652
  - oxidizing agents in, 696
  - pancake. See *Pancake flour.*
  - patent, composition, 629
  - peanut, 680–684. See also under *Peanut.*
  - proteases. See *Proteases.*
  - self-rising, composition, 629
  - soybean, 680–684. See also under *Soybean.*
  - wheat. See *Wheat.*
- Flower, regarded as vegetable, 731–732
- Fluidity, definition of, 399
- Flukes, 391
- Fluorine, 270, 290–291
  - in fish, 485
- Foam, 24, 39, 45–46
  - egg white, 45
  - milk, 46
  - whipped cream, 46, 422
- *Foeniculum vulgare*, 885

- Folestrin, 468
- Folic acid, 255
- Fondant, 816
- Food, carbohydrate content, 783
  - chemistry of, 3, 5, 6
    - colloidal, 9, 35–47
    - physical, 9–48
  - dehydrated, 362, 373, 374
  - digestion of, 327–355
  - essential factors, 2
  - feeds, 271, 272, 469
    - ash, 272
  - functions, 2
  - infection vector, 389
  - machines and molds, 722
  - palatability, 8, 9
  - poisoning vector, 380–391
    - bacteria, 381–390
    - botulism, 381–385
    - chemical poisons, 381
    - parasites, 391
    - plants and animals, 381
    - salmonella, 388–390
    - staphylococcus, 385–388
  - preservation, 3, 5, 356, 370, 375–379. See also under *spoilage* below and individual foods.
    - by chemicals, 377
  - processing, 3, 6, 7
  - spoilage of, 356–380
    - and accessory growth factors, 370
    - and acids, 367, 368
      - amino, from proteins, 360
      - from carbohydrates, 357–359
      - from fats, 360
    - analysis for, 379–380
      - bacteriological, 379
      - chemical, 380
      - organoleptic, 379–380
    - biochemical factors in, 357–362
    - changes involving synthesis, 361
    - and chemical environment, 367–370
    - conditions affecting, 363–373
    - by enzymes, 361–362, 373–374
    - fermentation in, 358–359
    - and gaseous environment, 371
      - carbon dioxide, 372
      - oxygen, 371–372
    - initial contamination, 366–367
    - microbial, 361, 363–373. See also under *Bacteria.*
    - and moisture content, 372–373
    - oxidative changes, 362, 374–375
    - prevention of, 375–378
      - dehydration, 378
      - hermetically sealed containers, 378
      - pasteurization, 376–377
      - refrigeration, 375
      - sanitation, 378
      - sterilization, 375–376
      - use of chemicals, 369, 377
    - and proteins, 359–360
    - proteolysis in, 358–359
    - sodium chloride in, 368–369
    - and storage, 364–365

Food (contd.)
    - and sugar, 369
    - and temperature, 363–364
- Foodstuffs. See also *Food.*
  - definition, 271
- Formaldehyde, 51, 76, 153
  - in fish, 492
- Formol titration, 153
- Fortification, by minerals and vitamins, of bread, 297–298, 711
  - of flour, 662–667
- Fowl, 525. See also *Poultry.*
- *Fragaria chiloensis*, 763
- Frankfurter, 104
- Froth. See *Foams.*
- Fructopyranose, 791
- Fructosazone, 83
- Fructose, 12, 64, 349, 350, 690
  - in banana, 776
  - in beet, 748
  - in coconut, 762
  - in cucurbits, 730, 731
- α-γ-D^--Fructose, 61
- β-γ-D^--Fructose, 61
- β-Δ-D^--Fructose, 61
- D^--Fructose, 51, 60, 61, 68, 85, 791. See also *Levulose*
  - and D^+-galactose, 794
  - and glycogen, 796
  - in inulin, 787
  - in stachyose, 788
  - in sucrose, 790
- *dl*-Fructose, 76
- Fructose compounds, diphosphate, 800, 801
  - monophosphoric acid, 800
- Fruit(s), 3, 10, 23, 43, 51, 52, 105, 252, 295, 762–781
  - berries, 762–765
    - blackberry, 28, 764, 778
    - blueberry, 765, 778
    - cranberry, 765. See also *Cranberry.*
    - gooseberry, 28, 765, 778
    - huckleberry, 765, 778
    - loganberry, 764, 778
    - Persian berry, 301, 324
    - raspberry. See *Raspberry.*
    - strawberry, 763. See also *Strawberry.*
  - citrus, 766–769
    - citron, 769, 778
    - grapefruit, 767–768. See also *Grapefruit.*
    - lemon, 767. See also *Lemon.*
    - lime, 28, 768
    - orange, 766. See also *Oranges.*
    - tangerine, 767
  - composition of, 778–779. See also under individual fruits.
  - drupes, 769–772
    - apricot, 769, 778
    - cherry, 769–770. See also *Cherry.*
    - peach, 770–771. See also *Peach.*
    - plum, 771–772. See also *Plum* and *Prune.*
  - grape, 772–773. See also *Grape.*
  - melons, 773–774, 779
  - persimmon, 783
  - pomes, 774–776
    - apple, 774–776. See also *Apple.*

Fruit(s) (*contd.*)
    pear, 776. See also *Pear.*
    quince, 776, 779
  preserved, 52
  ripening by ethylene, 767, 773, 776
  (sold as) vegetables, 732–737
    corn, sweet, 733–736. See also *Corn, sweet.*
    eggplant, 732, 757, 783
    garden pepper, 732–733. See also *Peppers.*
    gumbo, 733
    okra, 733, 757, 783
    tomato, 736–737. See also *Tomato.*
  tropical, avocado, 776, 778, 783
    banana, 777. See also *Banana.*
    date, 779–780. See also *Date.*
    fig, 780. See also *Fig.*
    pineapple, 780–781. See also *Pineapple.*
    pomegranate, 779, 781, 783
Fruit acids as confectionery flavors, 836
Fruit juices, 22, 34, 47, 52, 358
Fryer, 526. See also *Chicken.*
Fuchsin, fast acid, 302
Fucosterol, 99
Fucoxanthin 492
Fumaric acid, 204
Fumaric hydrogenase, 217, 223
Fungi, 51, 98. See also *Mushrooms.*
  edible, 756–759
Fungicides, 52
Furane ring, 820
Furanoid ring, 61
Furfural, 84
Fusel oil, 84
Fustic, 322

G

Gabriel synthesis, 129
Gadoleic acid, 90, 565
Gadus
  callarias, 488
  macrocephalus, 488
  morrhua, 486
Galactan, 68, 793
  in coconut, 762
  in legumes, 743, 745
Galactolipid, 89, 103
Galactopyranose, 793, 794
Galactosan, 68
Galactose, 58, 81, 89, 103, 349, 350, 762, 820
  and glycogen, 796
  in hemicellulose, 786
  in raffinose, 788
α-*D*-Galactose, 72
D^+-Galactose, 51, 55, 56, 58, 68, 70, 78, 85, 791, 793–797
  and D^--fructose, 794
  and D^+-glucose, 793, 794
  in lactose, 789
  series, 55–56
L^--Galactose, 55, 56, 58, 70
4-β-Galactosylglucose, 64
4-β-D^+-Galactosyl-D^+-glucose, 62, 64
Galacturonic acid, 820
  in gum arabic, 822
Galeorhinus zyopterus, 489
Gall bladder, function in digestion, 327, 328
Gallotannin, 318
Gallstone, 100, 278
Garblers, 3
Garlic, 729
  composition, 757
Gastrin, 332
Gastropod, 99, 177
Gaylussacia resinosa, 765
Gel, 36, 37, 38, 39–40, 41. See also *Colloids.*
Gelatin, 33, 36, 42, 44, 47, 104, 114, 117, 126, 128, 164, 179, 200, 209, 467–468, 619, 823–824
  amino acids in, 126, 619, 824
  plain, dry, composition, 812
Gelidium, 822
Gelose, 822
Gentian, 319
Gentiana lutea, 319
Gentisin, 319
Geraniol, 766
Germ, 599, 600, 601, 602, 621, 623, 626
Germicides, 718
Germo alalunga, 489
Ghatti, 819
Ghee, 424
Giardia lamblia, 391
Ginger, 886–887
  limed, 887
  Jamaica, 887
  standards, 886
Ginger ale, *p*H of, 28
Gingerol, 886
Gioddu, 432
Glassine, waxed, 726
Gliadin, 112, 117, 128, 162, 178, 179, 283, 617, 618, 620, 647, 650
Globin, 112, 114, 117, 175
  denatured, 176
Globulin, 111, 128, 174, 178, 617, 618, 619, 647, 681
α-Globulin, 619, 681
  in Lima beans, 744
β-Globulin, 619, 681
  in legumes, 745, 747
  in Lima beans, 744
  in nuts, 759, 761
  in potatoes, 756
Glucodecose, 68
Glucoheptose, 68
Gluconic acid, 65, 78, 81, 84
D^+-Gluconic acid, 65, 77
Gluconolactone, 81
D^+-Gluconolactone, 77
γ-D^+-Gluconolactone, 65
Glucononose, 68
Glucooctose, 68
Glucoprotein, 167
Glucopyranose, 792, 793
Glucosazone, 83
Glucose, 9, 12, 34, 58, 65, 81, 108, 142, 143, 199, 225, 226, 259, 273, 322, 348, 349, 350
  in coconut, 762
  commercial, 51
  in cucurbits 730, 731

Glucose (contd.)
  cyanohydrin, 79
  formation, 68
  fruits, 768, 770, 776
  and glycogen, 796
  liquid, 816
  sirup, 816
  in vegetables, 738, 748
α-Glucose, 208
α-β-D^+-Glucose, 82, 83
  system, 73
α-*D*-Glucose, pyranoid ring formula, 60
α-D^+-Glucose, 60, 65, 78, 816
α-L^--Glucose, 65
β-D^+-Glucose, 60
  in cellulose, 786
D^+-Glucose, 51, 54, 56, 58, 61, 64, 65, 68, 70, 73, 77, 79, 80, 83, 84, 85
  catabolism of, 797
  in cellulose, 786
  in dextrins, 788
  formula, constitutional, 55
    empirical, 59
    molecular, 54, 59
    semiconstitutional, 54, 59
    semistructural, 59
    skeletal, 54, 55, 59
  and D^+-galactose, 783, 787, 788, 792, 793, 794
  in glycogen, 788, 798
  and lactic acid, 798–799
  in lactose, 789
  in maltose, 790
  series, 56
  in starch, 787
  in sucrose, 790, 791
L^--Glucose, 56, 58, 61, 65, 70
L^+-Glucose, 58
Glucose compounds, -*l*-phosphate, 204
  monophosphoric acid, 800
Glucoside, 80, 103, 254, 887, 888
  mono-, in grapes, 772, 773
  in oranges, 766
Glucosidoprotein, 114
α-Glucosyl-β-γ-fructoside, 68
α-D^+-Glucosyl-β-γ-D^--fructoside, 64
4-α-Glucosylglucose, 64
4-α-D^+-Glucosyl-D^+-glucose, 62
4-β-Glucosylglucose, 64, 68
4-β-D^+-Glucosyl-D^+-glucose, 62
Glucuronic acid, 80
Glue, animal, 470
Glutamic acid, 9, 117, 120, 124, 125, 128, 131, 132, 133, 135, 142, 143, 149, 154, 155, 158, 164, 180, 527, 618, 743, 747
d-Glutamic acid, 128
Glutamic dehydrogenase, 199, 218
Glutamine, 142, 352
Glutamylcysteylglycine, 138
Glutathione, 139, 144, 149, 150, 185, 225, 623
Glutelin, 111, 617, 618, 620, 647, 681
Gluten, 10, 75, 117, 128, 620, 647, 650, 651–652, 654, 694, 695, 697, 702
  creaming power, 703
  elasticity, 697
  feed, calcium content, 279
Gluten (contd.)
  oxidizing agents, influence on, 652
  proteins, 22, 694, 695, 702. See also *Glutenin.*
Glutenin, 22, 111, 117, 179, 618, 620, 647, 650
Glyceraldehyde, 49, 64, 76, 105, 143, 803, 808, 809
D^+-Glyceraldehyde, 50, 51, 64, 67
dl-Glyceraldehyde, 76
L-Glyceraldehyde, 64
Glyceric acid, 143
D^+-Glyceric acid, 800
Glyceride(s), 89–96, 211, 339, 351, 564, 566, 567, 575, 580, 703. See also *Fatty acids.*
  in cabbage, 737
  chemical behavior, 92–95
  nomenclature, 92
  in nuts, 760, 761, 762
  physical properties, 92–95
  rule of even distribution of, 91
  in soybean oil, 745
  structure, 89–92
  synthetic, 95
Glycerol, 84, 88, 92, 93, 97, 98, 100, 102, 105, 470, 487, 737, 809
β-Glycerophosphate, 209
Glycerophosphoric acid, 101
Glycerophosphoric enzyme, 218
Glycerose, 70
Glyceryl compounds, esters, 92
  palmitate-oleate-stearate, 808
  trinitrate, 97
  tristearate, 92
  tristearin, 92
Glycine, 51, 111, 117, 119, 124, 125, 128, 130, 133, 136, 139, 142, 143, 144, 146, 149, 154, 155, 156, 180, 255, 258, 345, 352, 527, 618, 743, 747
  anhydride, 131
Glycinin, 179, 619, 681
  in soybean, 745
Glycogen, 51, 68, 75, 78, 85, 204, 788, 792
  anabolism of, 142, 796–797
  catabolism of, 85, 797–806, 808
  in fish, 492
Glycogenase, 85, 758, 798
Glycogenesis. See *Glycogen, anabolism.*
Glycogenolysis. See *Glycogen, catabolism.*
Glycolaldehyde, 49, 50, 51, 67, 76, 78, 143
Glycolic acid, 143
Glycolipids, 89, 103
Glycoproteins, 112, 114, 167, 172–174, 329
Glycosides, 65, 68, 80
Glycosidic linkage, 62, 82
Glycuronic acid, 173, 174
Glycylalanine, 136, 146
Glycylalanine diketopiperazine, 136
Glycylglycine, 135, 136
Glycylleucine, 198, 344
Glycyltyrosine, 194
Glyoxalase, 150
Glyoxylic acid, 152
Goat milk, 90, 403, 412
Goiter, 287
Golden sirup, 817
  composition, 818

Gooseberry, 28, 765
  canned, composition, 778
  composition, 778
Grains, 10, 252
  brewers', composition, 631
  cereal. See *Cereal grains.*
  distillers', composition, 631
Gramicidin, 370
Gramineae, 592
Grape, 27, 28, 772–773
  American, 772
    composition, 778
    ripening, 772
    types, 772
  black, 318
  carbohydrate content, 783
  European, 772–773
    composition, 778
    muscat, 772, 773
      composition, 773
    Tokay, 772, 773
    types, 772
  juice, 47
Grapefruit, 28, 767–768
  carbohydrate content, 783
  composition, 778
  pulp, composition, 768
Grass juice factor, 255
Grayfish, 489
Grease oil, 470
Green, fast, FCF, 303, 315–316, 317
  Guinea, B, 303, 313–314, 315
  light, SF yellowish, 303, 314–315, 316, 317
  malachite, 314
  naphthol, B, 303
Grenadine, 781
Groats, buckwheat, 630
  oat, 602, 638
Groundnut oil, 90
Groupers, 478, 500
Growth factors, accessory for bacteria, 370–371. For individual factors, see under name of factor.
Guanidinoacetic acid, 260
Guanidobutylamine, 143
Guanilic acid, 169, 170
Guanine, 167, 168, 172
Guilds, 3, 4
Guinea green B, 303, 313–314, 315
Guinea pig factors 1, 2 and 3, 255
Gulose, 59, 78
D^--Gulose, 56, 61, 70
L^+-Gulose, 56, 58, 61, 70, 80
  by inversion, 58
Guluronolactone, 81
Gum(s), 22, 42, 52, 79, 819–823
  agar agar. See *Agar-Agar.*
  arabic, 42, 51, 52, 813, 815, 819, 821–822
  carbohydrate, 647
  ghatti, 819
  Irish moss, 819
  Karaya, 819
  locust kernel, 819, 823
  mesquite, 68
Gum(s) (contd.)
  pectin, 819–921. See also *Pectin.*
  pectinlike, 647
  quince seed, 819
  tragacanth, 52, 822
Gumbo, 733
Gymnosarda pelamis, 489

H

Haddock, 475, 476, 483, 484, 486, 488, 491, 500, 510, 513
Haematoxylin, 318, 323
Haematoxylon campechianum, 318, 323
Hair, 110, 112
Hake, 475, 500, 513
Halibut, 104, 475, 478, 479, 480, 481, 483, 484, 488, 500, 619
  muscle protein, 619
Halphen test, 576
Ham, 279, 445, 452, 461
  asphalt-coated, 461
  quick-cured, 460
  souring of, 460
  types, 460
Hapten, 150, 151, 177
Harden-Young ester, 799
Hay bacillus, 719
Heart, 100, 126, 169
Hehner value, 573
Heifer, 442
Helianthus tuberosus, 756
Helicoprotein, 112
Helicorubin, 112, 175
Helix pomatia, 112
Helminths, 391
Hematin, 112, 175, 285, 286
Heme, 175, 176
Hemicellulose, 600, 786
  in navy bean, 743
  in soybean, 745
Hemin, 175, 216, 219, 220
  type of protein, 216
Hemochromogen, 176
Hemocyanin, 111, 162, 177, 217
Hemoglobin, 112, 114, 117, 159, 164, 175, 185, 209, 216, 219, 285, 289, 345, 454, 455, 458, 459
  and copper, 289
  formation, 286
  horse, 162
  and iron, 285
  man, 162
  oxygenation of, 545, 456
  reduced, 449, 450
Hemp, globulin of, composition, 117
  oil, linolenic acid in, 91
Hempseed, 111, 178
Hen. See *Egg, Chicken.*
Heparin, 174
Heptacosanol, 774
n-Heptanoic acid, 54, 79
Herring, 480, 491, 492, 510
  kippered, 510
  lake, 480, 484, 486, 513
  oil, 90, 91

Herring (contd.)
  sea, 475, 483, 484, 510, 513
  sperm, 112
Hesperidin, 254, 766
Hexanal, 807
Hexestrol, 264
Hexokinase, 799
Hexopyranoses, α-*D*-series of, 71–72
Hexosamine, 173
Hexosan, 68
Hexose, 51, 58, 64, 68, 73, 85, 98, 108
  -phosphoric acid equilibrium, 799
  sugars, 612
  unit of gums, 819
Hexose compounds, diphosphate, 801, 802, 806
  diphosphoric acid, 799
  monophosphate, 217, 223, 806
  monophosphoric acid, 800
  phosphates, 350
  phosphoric esters, 800
  pyranoses, 73
Hexosemonophosphoric dehydrogenase, 218, 223
Hickory, 759–760
  nut, 759
    composition, 778
Hides, 471. See also *Skin.*
Hippulin, 263, 264
Hippuric acid, 130, 142
Hisbiscus esculentus, 733
Histidine, 117, 120, 125, 126, 127, 128, 130, 131, 132, 133, 135, 141, 143, 145, 149, 152, 153, 154, 155, 158, 164, 165, 257, 258, 354, 468, 484, 527, 618, 621, 645, 742, 743, 744, 745
Histohematin, 217
Histone, 112, 117, 128, 177
Hog, razor-back, 435, 437
  typical, 437
Hominy, 28, 633, 635
  carbohydrate content, 783
  composition, 629, 631
  feed, composition, 631
  mineral content, 630
  pearled, composition, 631
  vitamin content, 630
Homocysteine, 143, 155, 260
Homocystine, 143, 155
Homogenization, 43, 46, 400, 416, 417
Homogenizer, 42, 43
Homogentisic acid, 143
Honey, 23, 47, 51, 52, 810, 818
  in candy, 814
  carbohydrate content, 783
  composition, 281, 812, 818
Honeybee, 818
Honeydew melon, composition, 779
Hopkins-Cole reaction, 152, 156
Hordeae, 592
Hordein, 112, 162, 179, 618
Hordeum
  distichon, 592
  vulgare, 592
Hormone(s), 224, 226, 229, 260–269
  acetylcholine, 269
  adrenal, cortical, 262, 266, 267, 353
Hormone(s) (contd.)
  androgenic, 262–263
  angioxyl, 269
  carotidin, 269
  of corpus luteum, 263, 265
  corticotropic, 262
  and enzymes, 224–226
  estrogenic, 263–265
  follicular, 263
  gastric, 332
  gonadotropic, 261
  hypophyses, 226
  kallikrein, 269
  lactogenic, 262
  luteinizing, 261
  medullar, 267
  padutin, 269
  pancreatic, 262, 268
  parathyreotropic, 261, 277
  pineal, 269
  pituitary, 261–262
  progesterone, 265
  protein, 148
  renin, 269
  secretin, 269
  thymus, 269
  thyroid, 226, 267–268
  vagotonine, 269
  and vitamins, 224–226
Hormozyme, 226
Horse-radish, 177, 887
Horse serum, 150
  albumin, 150
Huckleberry, 765
  carbohydrate content, 783
  composition, 778
Hulls, buckwheat, 630
  corn, 633
  oat, 638
  rice, 642, 643, 645
Humin, 118
Hydantoic acid, 140
Hydantoin, 130, 131
Hydnocarpic acid, 90
Hydrazone, 82
Hydrochloric acid, 26, 27, 28, 30
  carbonic anhydrase theory, 334–335
  in gastric juice, 333, 334, 335
  secretion of, 334, 335
Hydrogenation, 93, 568
  of cottonseed oil, 576
Hydrogen ion, 11, 25–35. See *p*H.
  activity, 25, 26, 25–35
  concentration, 25–26, 27, 29, 40, 188
  method of expression, 26, 27
Hydrogenolysis, 93, 94, 98
  catalytic, 79
Hydrogen peroxide, 192, 194
Hydrogen sulfide hemoglobin, 458, 459
Hydrolagus colliei, 489
Hydrometer, 11, 12
  Balling, 12
  Brix, 12
Hydroquinone, 94

- Hydroxamic acid, 94
- Hydroxyacetaldehyde, 49
- Hydroxyamino acid, 102
- Hydroxyaspartic acid, 123
- β-Hydroxybutyrate, 107
- β-Hydroxybutyric acid, 106, 143
- Hydroxybutyric acid dehydrogenase, 223
- 17-Hydroxycorticosterone, 266
- 17-Hydroxydehydrocorticosterone, 266
- 17-Hydroxydesoxycorticosterone, 266
- Hydroxyethanal, 49
- Hydroxyglutamic acid, 133, 154, 180
- β-Hydroxyglutamic acid, 117, 121, 125, 132, 134, 142
- Hydroxyl ion, 26, 29
- Hydroxylysine, 123
- Hydroxymethylfurfural, 85
- (Hydr)oxynervone, 89, 103
- (Hydr)oxynervonic acid, 89, 103

I

- *Illicium verum*, 881
- Illipe butter. See *Tallow, Borneo.*
- Imbibition, 40–41. See also *Colloid.*
  - hysteresis, 41
  - syneresis, 40–41
- *dl*-β-4-Imidazolelactic acid, 179
- β-Imidazolethylamine, 142
- Indican, 318
- Indigo, 322
- Indigo carmine, 303, 317, 318
  - reactions, 317
  - spot tests, 318
- *Indigo ferae*, 318, 322
- Indigotin, 303, 317, 318, 322
- Indole, 145, 152, 153, 354, 360
- Indoleëthylamine, 145
- β-Indoleëthylamine, 143
- Indolepyruvic acid, 143
- 3-Indolepyruvic acid, 179
- Indophenol oxidase, 176, 226
- Indoxyl, 284
- Infant foods, 52
- Inhibitols, 94
- Inhibitors, of enzymes, 202–208, 215
  - competitive, 203, 205
  - noncompetitive, 203, 205
- Inosinic acid, 169, 170, 800, 801
- Inosite, 766, 768
- Inositol, 230, 243, 258
- Insulin, 117, 148, 149, 162, 222, 225, 262, 268, 787
  - and glycogen, 797, 798
- Interfacial tension, 11, 23, 38, 43, 44, 580
- Intermedin, 261
- Intestine, 198
  - casings, 468
  - digestion in, 337–346
  - enzymes in, 342–344
  - large, function of, 353–355
    - putrefaction products in, 354
  - putrefaction in, 143–146, 354
  - secretions of, 341–343
  - small, absorption by, 346–353
- Inulin, 51, 79, 731, 756
- Invalid foods, 52
- Inversion, 58, 66
- Invertase, 349, 795, 796
- Invert sugar, 813, 816, 818
- Iodine, 270, 286–288, 464, 626
  - in fish, 485, 486
  - and goiter, 287
  - sources in foods, 288
- Iodine number. See *Iodine value.*
- Iodine value, 94, 572, 581, 582, 584, 585
  - of cereal oils, 622
  - of shortening, 587
- Iodoacetic acid, 349, 350, 353
- Iodogorgoic acid, 32, 121, 148, 268
- *Ipomoea batatas*, 749–751
- Irish moss, 819
- Iron, 270, 275, 276, 278, 284–286, 297, 298, 464
  - and anemia, 285–286
  - available in foods, 286
  - in berries, 763, 764, 765
  - in bread, 711, 712
  - in cereal grains, 626, 627, 630, 637, 644, 656
  - in cucurbits, 730, 731
  - in fish, 485, 486
  - in flours, 682
    - enrichment, 662–667
  - in fruits, 740, 766, 767, 768, 769, 770, 771, 772, 773, 775, 776, 777, 780, 781
  - and hemoglobin, 285
  - inorganic forms, 285
  - in legumes, 743, 744, 745, 747
  - organic forms, 285
  - in peanut, 760
  - in vegetables, 731, 732, 733, 734, 737, 738, 739, 742, 748, 749, 751, 752, 756
- *Isatis tinctoria*, 318
- Isbell method, 78
- Isoamylamine, 143
- Isocitric dehydrogenase, 218, 223
- Isoelectric point, 33, 40, 114, 213
- Isoleucine, 117, 120, 126, 127, 132, 133, 134, 142, 154, 180, 257, 258, 618, 621
- Isomerization, 59, 85
  - geometric, 99
- Iso-oleic acid, 581
- Isotope, heavy, in study of metabolism, 181–182
- Isovaleric acid, 90, 143

J

- Jack bean meal, 214
- Jam, 28, 34
  - manufacture, 52
  - substitutes, 52
- Jellies, 23, 28, 34
- Jellying power, 23
- Jerusalem artichoke. See *Artichoke.*
- *Juglans*
  - *cinerea*, 761
  - *nigra*, 761
  - *regia*, 761
- Juglansin, 761

K

- Kafirin, 112
- Kale, 280

Kale (*contd.*)
  calcium content, 279
  carbohydrate content, 783
Kallikrein, 269
Kapok oil, 576, 584
  characteristics, 582
  composition, 581
Karaya, 819
Katsuwonus pelamis, 489
Kefir, 431
Kerasin, 89, 103
Keratin, 112, 117, 128, 166
α-Keratin, 166
β-Keratin, 166
Ketogenesis, 106, 142
α-Ketoglutaric acid, 143
Ketohexose, 49, 68, 791
Ketohydrazone, 82
α-Keto-β-hydroxypropionic aldehyde, 143
Ketokinase, 800
Ketone, 77
Ketone bodies, 107
Ketonuria, 106
Ketopentose, 49, 68
2-Ketopropanal, 803
Ketose, 60, 65, 67, 79, 81
Ketosis, 106
Ketosuccinic acid, 143
Ketotetrose, 49, 65
Ketotriose, 49, 67
Kidney, 51, 100, 465
Kiliani synthesis, 81
Kinase, 203
Kirschner value, 573, 583
Kitol, 234
Knoop theory, 106, 107
Kohlrabi, 739
  carbohydrate content, 783
  composition, 281, 757
Koumiss, 431
  carbohydrate content, 783
Kynurenic acid, 143, 145
Kynurenine, 143, 145

L

Laccase, 217, 222
Lactalbumin, 111, 117, 162, 178, 398, 402, 406, 619
Lactase, 85, 342, 349, 796
Lactic acid, 51, 52, 84, 108, 143, 150, 349, 398, 403, 406, 698, 699, 712, 717, 801, 802
  concentration in blood, 805
  cycle, 806
  in fish, 492–493
  formation from D^+-glucose, 798–799
  formation from glycogen, 798, 804
  intermediates, 808
  in muscle, 798
  oxidation of, 802–803
β-Lactic acid, 143
L^+-Lactic acid, 800
Lactic dehydrogenase, 199, 218, 223
Lactobacillus
  acidophilus, 788, 789
  casei, growth factor, 255
Lactoflavin, 237
Lactoglobulin, 162, 406
Lactol, 65
  formation, 66
Lactone, 65, 81
  formation, 66
γ-Lactone, 65
Lactose, 51, 52, 53, 62, 64, 68, 75, 78, 85, 349, 398, 404, 569, 691, 789
α-Lactose, 789
β-Lactose, 789
Lactuca sativa, 739
Lamb, 437, 439
  chops, broiled, 104
  classes and grades, 444
  composition, 279, 446
  consumption, 439, 440
  production, 439
Langenbeck cycle, 224
Lanosterol, 99
Lard, 104, 466, 527, 565, 566, 567, 569, 570, 571, 576, 577, 580, 584, 586, 588, 703
  characteristics, 582
  composition, 581
  iodine value, 584
  for shortening, 703
  synthetic, 93
Lard oil, 90, 95, 590
Laurel oil, 90
Lauric acid, 43, 90, 565, 573, 582, 762
Lauryl alcohol, 98
Lead, 278, 485
  in coloring matters, 318
Leavening, 661, 700–701, 705, 712
  of cake, 700–701
Leaves and stems, vegetables which are, 737–742
  Brussels sprouts, 737
  cabbage, 737–738
  celery, 738
  Chinese cabbage, 739
  kohlrabi, 379
  lettuce, 739
  parsley, 739–740
  rhubarb, 740
  spinach, 741–742
  Swiss chard, 742
Lecanora tartarea, 319, 323
Lecithin, 47, 89, 100, 101, 102, 112, 174, 177, 345, 569, 584, 621, 745
  beef liver, 101
α-Lecithin, 101
Lecithoprotein, 112, 167, 177
Lecithovitellin, 112
Leghorn, 524
Legume, 295, 620, 742–747. See also under individual legume.
Legumelin, 111, 179, 619, 745, 747
Legumin, 111, 179, 619, 746, 747
Lemon, 28, 767
  carbohydrate content, 783
  composition, 778, 812
  extract, 896
Lemon oil, 896, 897
  terpeneless, 897

Lens esculenta, 746
Lentil, 746
  dry, composition, 757
Leprotene, 230
Leptocephalus conger, 489
Lettuce, 251, 739
  carbohydrate content, 783
  composition, 757
Leucine, 117, 120, 124, 125, 126, 127, 128, 130, 132, 133, 134, 142, 143, 149, 154, 156, 180, 257, 258, 527, 618, 621, 743, 747
  aminopeptidase, 343
d-Leucine, 182
l-Leucine, 182
Leucoflavin, 239
Leucomalachite green, 194, 199
Leucosin, 111, 117, 618, 620
Leucyldiglycine, 343
l-Leucylglycine, 216
Leuichthys artedi, 486
Levulans, 68
Levulose, 61, 68, 750, 818. See also *D^--fructose*.
Liebermann (or Liebermann-Burchard) reaction, 99
Life expectancy, 804
Lifschutz reaction, 99
Ligature, 472
Light green SF yellowish, 303, 314–315, 316, 317
Lignin, 621
Lignoceric acid, 89, 90, 102, 103, 565, 745, 760
Lignocerylsphingomyelin, 102
  fatty acid ester, 102
Lima bean. See *Bean, Lima*.
Lime, 28, 768
  in citrus fruits, 767
  composition, 778
  in cranberries, 765
Lime water, 27, 28
d-Limonene, 881, 882, 885, 897
Linoleic acid, 91, 92, 94, 256, 464, 564, 573, 581, 582, 622, 737, 745, 759, 760, 761
Linolein, 93, 95
Linolenic acid, 91, 94, 256, 487, 890
Linoleyl alcohol, 98
d-Linolool, 884
Linseed oil, 91, 94, 95, 581, 584
  characteristics, 582
  composition, 581
Lipase, 92, 198, 202, 211, 334, 339, 350, 351, 362, 527, 795
  gastric, 335
  liver, 194
  pancreatic, 194, 339
  unit, 208
Lipid(s), 9, 39, 88–109, 150, 151, 339, 345
  absorption of, 350–352
  amino-, 89
  in cereals, 621
  cerebrosides, 103
  classification, 88–89
  complex, 88, 100–103
  fats and oils, 89–98
  galacto-, 89, 103
  glyco-, 89, 103
  hydrolysis of, 92, 341
Lipid(*s*) (*contd.*)
  metabolism of, 105–107
  in nutrition, 103–109
  phospho-, 88, 100–103, 105, 177, 350, 351, 487
  simple, 88, 89–100
  sterols, 98–100
  sulfo-, 89
  synthesis of, biological, 107–109
  unsaponifiable matter of, 93, 98
  waxes, 98–100
Lipin, 88
Lipoid, 88. See *Lipid*.
Lipoprotein, 177
Lithium, 485, 626
Lithocholic acid, 345
Litmus, 319, 323
Liver, 100, 105, 112, 126, 149, 169, 198, 199, 252, 465
  beef. See *Beef, liver*.
  in digestion, 327, 328
  and glycogenesis, 797
  and glycogenolysis, 797, 798
  preparations, 468
  tissue, 75
    of pigs, 91
Lobster, 475, 492, 516, 518
  canned, 104
  spiny, 479
  whole, carbohydrate content, 783
Locust kernel, 819, 823
Loganberry, 764
  canned, composition, 778
  composition, 778
Logwood, 318, 323
Lumisterol, 100, 247, 248
Lupin, 179
Lutein, 320
Lutianus blackfordii, 486
Lycopene, 320, 736
Lycopersicum esculentum, 736
Lyophile. See *Colloids*.
Lyophobe. See *Colloids*.
Lysine, 112, 117, 120, 125, 126, 127, 128, 131, 132, 133, 135, 143, 145, 149, 153, 154, 155, 158, 164, 165, 175, 178, 257, 258, 340, 354, 484, 527, 618, 620, 621, 645, 680, 742, 743, 744, 745, 747
Lysozyme, 214
D^--Lyxose, 70
L^+-Lyxose, 70

M

Macadamia, 762
  composition, 778
Macadamia ternifolia, 762
Macaroni, 606, 671, 673–677
  carbohydrate content, 783
  composition, 629
  durum wheat and, 669, 671–672
  farina and, 662
  mineral content, 630
  vitamin content, 630
Macaroni products, 673–677
  alphabets, 674, 675

Macaroni products (*contd.*)
  definition, 673, 676
  long-goods, 674, 675
  noodles, 673, 674
  production and consumption, 674
  short-goods, 674, 675
  spaghetti, 673, 674, 675
  standards and definitions, 676–677
  types, 674–676
  vermicelli, 606, 673, 674, 675
Macaroon, 759
  carbohydrate content, 783
Mace, 889, 890
Mackerel, 112, 475, 476, 478, 479, 486, 489, 491, 500, 510, 513
  Boston, 484
  common, 483, 489
  king, 478
  oil content, 481
    effect of size on, 482
  Pacific, 489
  Spanish, 478, 484
  sperm, 112
Maclura tinctoria, 322
Maclurin, 322
Madder, 301, 318, 319, 323
Magnesium, 27, 28, 204, 216, 270, 278, 280–281, 295
  calcium, antagonistic action toward, 280–281
  in cereals, 626–627
  in fish, 485, 486
  in various flours, 682
  in foods, 281
  in milk, 407
Maize, 179, 591, 617, 632. See also *Corn*.
  botanical classification, 592
  oils, 90
  starch, 815
Majorana hortensis, 887
Malachite green, 314
  leuco compound, 194, 199
Malic acid, 143, 207
  in berries, 764, 765
  in fruits, 766, 769, 771, 774, 775, 776, 778–779
  in rhubarb petioles, 740
  in vegetables, 736, 742
l-Malic acid, in berries, 763, 764, 765
  in fruits, 769, 771, 772, 773, 774, 780
  in vegetables, 731, 739
Malic dehydrogenase, 218
Malol, 774
Malonic acid, 108
Malt, 51, 198, 623, 627, 790
  composition, 631
  diastatic, 687
  extract, 691, 695, 696
  production, 52
Maltase, 62, 73, 82, 198, 342, 349, 692, 758, 795, 796
  yeast, 194
Maltose, 51, 52, 53, 62, 64, 68, 75, 78, 194, 198, 200, 329, 330, 336, 339, 341, 349, 692, 693, 790, 792, 818
  in banana, 777
  in cereals, 790
  hydrolysis of, 795
Maltose (*contd.*)
  in sweet potatoes, 750
Malt sugar. See *Maltose*.
Manganese, 204, 216, 288
Mannan, 68
Manninotriose, 788
Mannite, 714, 732
Mannoic acid, 65
Mannosaccharic acid, 81
Mannosan, 68
Mannosazone, 83
Mannose, 58, 65, 81, 349, 350, 791
  in hemicellulose, 786
α-D-Mannose, 72
D^+-Mannose, 50, 56, 59, 61, 68, 70, 85, 791, 794
L^--Mannose, 56, 58, 70
Mannosemonophosphoric acid, 800
Maple sap, 51
  sirup, 47, 817–818
    composition, 812, 818
  sugar, 818
    composition, 812
Margarine, 47, 576, 579, 580, 585, 586, 588–589
  oils used for, 589
  types, 588
Marjoram, 887
Marmalade, orange, carbohydrate content, 783
Marshmallow, 816, 823
Martius yellow, 303, 310
Marzipan, 759
Maté, 873–874
Matzoon, 432
Maydeae, 592
Mayonnaise, 42, 43, 47, 580, 900–901
  standards, 900
Meat(s), 3, 34, 105, 192, 295, 435–473
  autolysis of, 447
  bloom, 449
  canned, 461
  casings, 468
  cattle breeds, 435
  classes, 441–445
  composition, 445–447
  compressed, 462
  consumption, 439
  cooking of, 462, 463
  curing of, 452–460
  definitions, Bureau of Animal Industry, 441
  dehydrated, 373, 461–462
  as food and feed by-products, 465–469
    animal feeds, 469
    fancy meats, 465
    fats, 465–467
    pet foods, 469
    pharmaceuticals, 468
  food poisoning from, 464–465
  freezing of, 451–452
  grades, 441–445
  inedible by-products, 470–472
    fat products, 470
    protein products, 470–472
  lean, 40
  livestock, raising and marketing, 435–438
  in nutrition, 464

- *Meat(s)* (*contd.*)
  - packing of, 438
  - post-mortem changes, 447–449
    - aging, 447–449
    - loss of bloom, 449
    - microbial growth, 449
    - rigor mortis, 447
    - shrinkage, 449
  - preservation of, 450–463
  - production, 439
  - products, 54, 435–473
  - proteins of, 126, 445, 446, 464
- Melanin, 118, 217
- *Melanogramus aeglefinus*, 486, 488
- Melissic acid, 90
- Melissyl alcohol, 98
- Melon, 773–774
  - honeydew, 779
  - musk-, 773–774
  - water-, 774
- Membrane potential, 33
- Menhaden, 476, 477, 478, 489
  - oil, 581, 584
    - characteristics, 582
    - composition, 581
- Mercaptan, 143, 354
- Mercury, 381
- *Merluccius bilinearis*, 488
- Mesocatalyst, 225
- Mesquite gum, 68
- Metanil yellow, 303
- Metaprotein, 113
- Methemoglobin, 221, 455, 456, 457, 459
- Methionine, 116, 117, 120, 125, 126, 127, 131, 132, 133, 143, 144, 154, 155, 158, 175, 179, 180, 182, 257, 258, 259, 260, 272, 283, 352, 354, 618, 621, 645
- *l*-Methionine, 128
- Methylamine, 143, 144
- Methyl butyrate, 198
- Methyl chavicol, 893
- Methylene blue, 204, 303
- Methyl α-D^{+}-glucose, 68
- α-Methyl-D^{+}-glucose, 60
- Methyl α-D^{+}-glucoside, 82
- Methyl β-D^{+}-glucoside, 82
- Methylglyoxal, 150
- Methyl group, essential transferable, 256, 259–260
- Methylmercaptan, 144, 354
- 2-Methyl-1,4-naphthoquinone, 253
- Methyl orange, 27
- Methylpentosan, 732, 760
- Methylprotocatechuic aldehyde, 895
- 17-Methyltestosterone, 262, 263
- Methyl tetramethyl-α-D^{+}-glucoside, 84
- Methyl tetramethyl-β-D^{+}-glucoside, 84
- Meyerhof-Kiessling concept, 802
- Michaelis constant, 202, 205
- Micronutrients. See *Elements, trace.*
- Milk, 3, 10, 27, 28, 30, 40, 42, 43, 47, 51, 52, 110, 112, 177, 178, 182, 185, 199, 210, 271, 272, 289, 295, 362, 365, 395–418
  - acidity of, 402, 403–404. See also *pH* below.
  - adhesion, 402
- *Milk* (*contd.*)
  - beverages, 434
  - boiling point, 399
  - buffer capacity, 403
  - in cake, 704, 705, 706, 709
  - certified, 414
  - churning, 46
  - coagulated, 113, 404
  - colligative properties, 399
  - composition, 404–413, 812
    - albumin, 117
    - amino acids, 619
    - carbohydrate, 405, 406
    - casein, 404, 619
    - differences due to breed, 406, 409, 410
    - enzyme, 405, 408
    - fat, 401, 404, 405, 410–412
    - gas, 405
    - lactalbumin, 619
    - lactose, 404, 405, 412
    - lipid, 402, 405, 406
    - mammalian, different species of, 412–413
    - mineral, 279, 288, 404, 405, 407, 412, 471
    - nitrogen distribution, 413
    - organic, 405
    - protein, 43, 47, 271, 404, 405, 406, 410–412, 619
    - serum, 27, 28
    - solids-not-fat, 404
    - total solids, 404
    - vitamin, 405, 408, 415
    - water, 404, 405, 409, 412
  - concentrated, 418, 425
  - condensed, 425
    - carbohydrate content, 783
    - composition, 426, 812
    - plain, 425
    - production, 396
    - sweetened, 425–426
  - conductivity, 400
  - consumption, 396
  - curd, tension of, 401
    - soft, 401, 416–417
  - definition, 398
  - dried, 432–433
    - in baking, 687, 689, 693
    - consumption, 397
    - production, 396
    - products, 432–433
      - composition, 433
    - skim, composition, 812
    - whole, composition, 812
  - evaporated, 424–425
    - composition, 425
    - consumption, 396
    - production, 396
  - expansion, coefficient of, 402
  - fermented, 431–432
  - filled, 433
  - fluidity, 399, 400
  - foam, 45, 46
  - freezing point, 399
  - frozen, 418
  - grades, 413–414
  - homogenized, 43, 400, 416, 417

- *Milk* (*contd.*)
  - human, 28, 412, 413
  - imitation, 434
  - malted, composition, 812
    - consumption, 397
    - powder, 433
    - production, 396
  - neutralizers, 400
  - odor, 398
  - osmotic pressure, 399
  - pasteurized, 414. See also *Pasteurization.*
  - *p*H, 27, 28, 402, 403
  - physical properties, 398–402
  - physicochemical properties, 402–404
  - production, 395
  - products, frozen, 427
  - reconstituted, 417
  - red, 719
  - refractive index, 400
  - sherbets, 427
  - skim, 43, 104, 401, 419, 420, 430, 431
    - carbohydrate content, 783
    - dry, phosphorus content, 279
    - specific heat of, 400
  - sour, 356, 358
  - specific gravity, 398
  - specific heat, 400
  - sugar. See *composition* above.
  - surface tension, 401
  - taste, 398
  - vitamin D, 415
  - vitamin-fortified, 415
  - wetting power, 402
  - whey. See *Whey.*
  - whole, carbohydrate content, 783
    - composition, 812
- Milk of magnesia, 27, 28
- Millon's reaction, 152
- Minerals, 2, 6, 9, 10, 270–299. See also *Ash*, *Elements*, and under individual element.
  - in animal body, group effects of, 294–296
  - in cereal foods, 630
  - in cereal grains, 626–627, 644
  - definition, 271
  - in fish, 485–487
  - in food, 270–299
    - preservation of, 369, 377
  - fortification with, 297–298, 662–667, 711
  - in meat, 464
  - in milk, 407
  - in nutrition, 270–299
    - absorption of, 348–349
    - acid-base balance, 295–296
  - in water, 295
- Moisture. See *Water.*
- Moistureproof wrapping, 706, 709, 721, 722, 726
  - cellulose, transparent, 726
  - glassine, waxed, 726
  - waxed, 726
- Molasses, 16, 27, 28, 47, 49, 817
  - Barbados, 817
  - blackstrap, 817
  - cane, carbohydrate content, 783
  - sirup, composition, 812, 818
- Molds, 34, 40, 134, 357, 360, 373, 719–723
  - and bakery products, 719–723
  - in cereal grains, 613
  - and fatty acids, saturated, 721
  - growth, conditions for, 720–721
  - prevention of, 722–723
    - by air conditioning, 723
    - by diacetates, 722
    - by ultraviolet radiation, 723
  - sources of, 722
  - spores, 720, 722
- Mollusks, 177
  - pulmonate, 175
- *Monilia*, 719
- Monoaminodicarboxylic acid, 120, 121
- Monoaminomonocarboxylic acid, 119, 121, 124, 125
- Monocalcium phosphate, 716, 717
  - in flour, 659, 660, 661
- Monoglyceride, 580, 703
- α-Monoglyceride, 96
- Mononucleotide, 168, 169
- Monopalmitodistearin, 576
- Monophenolase, 210
- Monophenol oxidase, 217
- Monophosphoric ester, 198
- Monosaccharide(s), 50, 51, 60, 61, 67, 78, 82, 349
  - aldohexoses, structure, 55–61
  - principles and history, 53–55
  - structure, 53–61
- Morin, 322
- Moroctic acid, 487
- Moss, Irish, 819
- Mouse factor, 255
- Mowrah fat, 92
- Mucic acid, 55, 80, 81
- Mucilage. See *Gums.*
- Mucin, 112, 114, 172, 173, 329, 334
- Mucoids, 114, 172, 173
- Mucoitinsulfuric acid, 173, 174
- Mucopolysaccharide, 173, 174, 329
- *Mucor*, 719
- *Mugil cephalus*, 486
- Mullet, 478, 483, 484, 486, 513
- *Musa sapientum*, 776
- Muscle, 110, 111, 126, 129, 199
  - glycogenolysis, 798
  - tissue, 51
- Mushroom, 217, 381, 756–759
  - composition, 757
  - enzymes in, 758
- Muskmelon, 773
  - carbohydrate content, 783
  - composition, 779
- Mussels, 246, 381
- Mustard, 47, 887–889
  - black, 888
  - composition, 889
  - flour, 888, 889
  - hulls, 888
  - oil, 888
  - prepared, 889
  - seed, oil, 90
  - standards, 889
  - white, 888

Mustelus californicus, 489
Mutarotation, 60, 67, 208
Mutton, 439
  classes and grades, 444
  consumption, 439, 440
  production, 439
  tallow. See *Tallow.*
Mycoderma
  aceti, 830
  cerevisiae, 712
Mycosterol, 98, 100
Myoglobin, 162
Myohemoglobin, 454
Myosan, 113
Myosin, 113, 464
Myosinge, 111
Myoxocephalus, 489
Myristic acid, 90, 487, 565, 581, 582, 760, 762
Myristica fragrans Houtt., 889
Myristicin, 890
Myristyl alcohol, 98
Myrosin, 887, 888
Myrtillin, 318
Myrtle wax, 90
Myxoxanthin, 230, 231

N

Nails, proteins in, 110, 112
Naphthalene, 142
Naphthol green B, 303
Naphthol yellow S, 309–310
  potassium salt, 303, 310
  reactions, 310
  sodium salt, 303
  spot tests, 310
Naphthylmercapturic acid, 142
Naringin, 836
Nasylates, 156
Neat's-foot oil, 470
Nectarine, 771
  composition, 779
Nematode, 391
Neothunnus macropterus, 489
Nerve, 110
  tissue, 100, 121
Nervone, 89, 103
Nervonic acid, 89, 103
Neuberg ester, 799
Neurogelatin, 126
Neuroplastin, 121
Neutralizer, cream, 400
  milk, 400
New coccine, 303, 305
Niacin, 224, 240–241, 297, 298, 464, 491, 492, 625
  in bread, 711, 712
  in cereal foods, 630
  in cereal grains and products, 625, 642, 643, 644, 646, 656, 657
  enrichment of flour, 662–667
  in various flours, 682
  in milk, 405, 407
Niacin amide, 221, 224, 230, 240–241, 255
Nickel, 270, 271, 293, 294, 485, 626
Nicotinamide. See *Niacin amide.*
Nicotinic acid. See *Niacin.*
Nicotinic acid amide. See *Niacin amide.*
Ninhydrin reaction, 141
Niobium, 485
Nitrate, 454
  in food preservation, 369, 377
Nitric oxide hemochromogen, 455, 458
Nitric oxide hemoglobin, 454, 455, 457, 458, 459
Nitrite, 454
  in food preservation, 369, 377
Nitrogen. See also *Amino acids* and *Proteins.*
  amino, Van Slyke method, 153
  formol titration, Sørensen method, 153
  in fruits, 775, 766
    lead tannin precipitate of oranges, 766
  isotopic, 181–182
  in rhubarb petioles, 740
  in spinach, 741
Nitrogen dioxide, 702
Nitrosyl chloride, 702
Noodles, egg, 674, 675, 676
  composition, 629
  definitions, 676
  production, 674
Norleucine, 121, 132, 133, 142, 154, 180
Norvaline, 123
Novadelox, 660
Nucleic acid, 112, 167, 168, 169, 172, 174, 219
Nucleohistone, 112
Nucleoprotein, 112, 167–172, 175, 219, 617, 620
Nucleosidase, 168, 795
Nucleoside, 168
Nucleotidase, 168, 795
Nucleotide, 170, 171, 221
Nucleus, 112, 168
Nulomoline, 813, 816, 818
Nutmeg, 889–890
  oil, 90
Nut(s), 252, 620, 759–762
  composition, 279, 759–762, 778–779
  domestic, 759–761. See also *Almond, Chestnut, Filbert, Hickory, Peanut, Pecan, Pinenut, Walnut.*
  tropical, 761–762. See also *Brazil nut, Cashew, Coconut, Macadamia, Pistachio.*

O

Oat flour, antioxidant properties, 637
  composition, 631
Oat meal, carbohydrate content, 782
  composition, 629, 631
  mineral content, 630
  vitamin content, 630
Oat(s), 595, 601–602, 610–611, 637–638
  amino acids in, 618
  black, 610, 611
  botanical classification, 592
  breakfast foods from, 678
  by-products, composition, 638
    definitions, 638
  classes, 610
  clippings, composition, 631
  composition, 615, 616, 631, 637
  gray, 595, 610, 611

- *Oat(s)* (*contd.*)
  - groats, 601
  - hulls, 51, 84
    - composition, 631
  - kernel, composition, 631
    - structure, 601–602
  - middlings, composition, 631
  - mill feed, composition, 631
  - mineral constituents, 279, 288, 626, 627
  - mixed, 610, 611
  - production, 593, 595
  - proteins in, 618
  - red, 592, 594, 610, 611
  - respiration, 614
  - rolled, composition, 629, 631, 637
  - standards, U. S. grain, 611
  - Tartary, 592
  - utilization, 637
  - vitamins in, 624, 625
  - white, 592, 594, 610, 611
  - yellow, 592, 594, 610
- Octadecapeptide, 137, 138
- Oenin, 318, 773
- *Oidium aurantiacum*, 719
- Oil red XO, 303, 307
  - reaction, 308
  - spot tests, 308
- Oil(s), 16, 88, 89, 92, 93, 94, 251, 564–590. See also *Fat(s)* and under source of individual oils.
  - acids derived from, 96–97
  - adulteration of, 576–577
  - antioxidants, 568
  - characteristics, 581–585
  - in coffee, 844
  - composition, 581
  - cooking, 579, 589
  - crude, evaluation of, 579
  - density, 582, 583
  - deterioration, tests for, 577
  - distinction between oils and fats, 567
  - drying, 94
  - edible, 564–590
  - examination of, 572
    - by chemical methods, 572–573
    - by physical methods, 573–574
  - fat composition, determination of, 575, 576
  - fatty acids, 564–566
    - separation of, 572, 573
  - fire point, 583
  - fish. See *Fish.*
  - flash point, 583
  - glycerides. See *Glycerides.*
  - Hehner value, 573
  - hydrogenated, 574, 577, 583, 587
  - iodine values, 95, 572, 581, 582
  - melting points, 92, 95, 582, 583
  - nature of, 564–570
  - neat's-foot, 470
  - nonglyceride components, 566
  - non-nutritional functions, 569–570
    - in baking, 570
    - in frying, 570
  - production, 571
  - rancidity, 577
- *Oil(s)* (*contd.*)
  - refractive index, 581, 582, 583
  - Reichert-Meissl value, 573
  - salad, 579, 589, 762, 900–901
  - saponification value, 95, 572, 581, 582
  - seed, flours from, 680–684
  - smoke point, 583
  - solubility of gases in, 583
  - sources, 570–571
  - special methods for finished products, 579–581
  - specialty, 589–590
  - specific heat, 583
  - specific, tests for, 576, 577
    - cottonseed, 576
    - peanut, 576
    - sesame, 576
    - tea seed, 576
  - stability tests, 577
    - Schaal method, 578
    - Swift method, 578
  - taste, contribution to, 570
  - tests for deterioration, 577
  - thiocyanogen value, 572
  - titer, 582, 583
  - unsaponifiable matter in, 95, 573, 582, 621, 622
  - utilization, 585, 586
    - for margarine, 588–589
  - viscosity, 583
- Okra, 733
  - carbohydrate content, 783
  - composition, 757
- Oleic acid, 90, 92, 97, 106, 351, 565, 573, 581, 582, 584, 622, 745, 759, 760, 762, 808, 890
- Olein, 93, 95
- "Oleo"-dipalmitin, 92
- "Oleo"-disaturated glyceride, 92
- "Oleo"-distearin, 92
- α-Oleo-distearin, 92, 564
- Oleomargarine, 762
- Oleo oil, 467, 569, 570, 585
- Oleostearine, 465, 570, 585, 587, 589
- Oleo stock, 467, 570, 585
- Oleyl alcohol, 98
- Oligosaccharide, 792
- Olive, 5, 28, 52
  - green, 104
    - ripe, carbohydrate content, 783
  - preservation of, 4, 5
  - pressing of, 4–5
- Olive oil, 4, 5, 95, 208, 351, 565, 570, 571, 576, 580, 584, 585
  - characteristics, 582
  - composition, 581
- *Oncorhynchus*
  - *gorbuscha*, 486, 488, 505
  - *keta*, 486, 488, 505
  - *kisutch*, 486, 488, 505
  - *nerka*, 486, 488, 505
  - *tschawytscha*, 486, 488, 505
- Onion, 728–729
  - composition, 757
  - fresh, carbohydrate content, 783
  - green, composition, 757
- *Oöspora*, 719

Ophiodon elongatus, 489
Optical rotation, 10, 17–19, 33
  internal compensation, 67
  polarimeter, 17, 18
  saccharimeter, 19
Orange, 28, 766
  carbohydrate content, 783
  composition, 776, 779, 812
  juice, 766
  mandarin, 767
Orange I, 303, 308, 312, 313
  reactions, 308
  spot tests, 309
Orange II, 303
Orange IV, 303
Orange G, brilliant, 312
Orange SS, 303, 309
  reactions, 309
  spot tests, 309
Orcein, 319, 323
Orchil, 319, 323
Orellin, 319
Organizer, 261
Organoleptic tests, 379–380
Ornithine, 142, 143, 204, 803
Oryzanine, 234
Oryza sativa, 592
Oryzeae, 592
Oryzenin, 620, 644
Osazone, 55, 60, 82, 83
  of lactose, 78
Osmotic pressure, 33, 36–37, 44, 159
  of milk, 399
Osseomucoid, 112, 173
Osteomalacia, 250
Ostrea
  gigas, 486
  lurida, 486
  virginica, 486
Ostreasterol, 99
Ovalbumin, 162, 178, 198, 619
Ovarian preparations, 468
Ovoglobulin, 111, 173
Ovomucin, 174
Ovomucoid, 173, 174
α-Ovomucoid, 174
β-Ovomucoid, 174
Ovovitellin, 112, 175, 178
Ox, muscle protein, amino acids in, 619
Oxalic acid, 381
  in blackberry, 764
  in vegetables, 732, 739, 740, 742
Oxidase. See *Enzymes*, *oxidative*, and under individual enzymes.
Oxidation, 357
  of carbohydrates, 80–81, 87
  of fats and fatty acids, 106–107
    β-theory, 106
    Knoop theory of, 106
    multiple alternate theory, 107
    ω-theory, 107
  and food spoilage, 361–362, 374–375
22-Oxidoergosterol, 246
Oxyhemoglobin, 220, 449, 454, 455, 457, 458, 459
Oxynervone, 103
Oxynervonic acid, 103
p-Oxyphenyl acetic acid, 144
p-Oxyphenyl propionic acid, 144
Oxytocin, 261
Oyster, 99, 475, 476, 477, 478, 484, 486, 492, 516–517
  carbohydrate content, 783
  glycogen in, 492
  iodine in, 288
  Pacific, 479, 486
Ozonolysis, 97

P

Padutin, 269
Palmitic acid, 90, 92, 106, 107, 487, 565, 581, 582, 737, 745, 759, 760, 761, 808, 890
Palmitin, 95, 102
Palmito-di-"olein," 92
Palmitoleic acid, 90, 565, 581, 582
Palm-kernel oil, 90, 565, 566, 570, 571, 573, 576, 588, 589
  characteristics, 582, 583
  composition, 582
Palm-nut oil, 90
Palm oil, 95, 566, 570, 571, 577, 584, 589, 590
  composition, 581
Pancake flour, 659, 661, 662
  buckwheat, 628
  wheat, composition, 629
Pancreas, 51, 126, 167, 169, 198, 338
  in digestion, 327, 328
  enzymes, 194, 338, 339–341, 528, 796
  hormones, 262, 268
  juice, 339, 345, 796
  secretion, 185, 337–338
    composition, 338
    control of, 337
    variations in, 338
Pansy, 318
Pantothen, 242. See *Pantothenic acid.*
Pantothenic acid, 183, 225, 230, 242, 255, 625, 626
  in cereal grains and cereal products, 625, 626, 643, 656, 657
  in various flours, 682
  in milk, 405, 407
Papain, 197, 198, 203, 209, 214, 344, 622
Papainase, 211, 216
Paprika, 892
  Hungarian, 892
  Koenigs-, 892
  Spanish, 892
  standards, 892
Paracasein, 335
Parathyroid, hormones of, 261, 277
Parnas concept of glycogen catabolism, 801
Parsley, 739–740, 890
  composition, 758
Parsnip, 749
  carbohydrate content, 783
  composition, 758
Pasteur enzyme, 216, 222
Pasteurization, 376–377, 414
  and milk foam, 46
  and viscosity, 400

Pastinaca sativa, 749
Pastries, 712, 762
  mold on, 720
Pea, 28, 111, 300, 746–747
  age and specific gravity, 746
  amino acids in, 619
  composition, 757
  canned, composition, 757
  carbohydrate content, 746, 783
  dry split, composition, 281, 288, 757
  green, 362
  proteins of, 179, 619, 746–747
Peach, 28, 770–771
  astringency, 771
  composition, 770, 771, 779, 812
  dried, composition, 779
  fresh, carbohydrate content, 783
  pressure test, 771
Peanut, arachin, 619, 681
  carbohydrate content, 783
  composition, 812
  conarchin, 619, 681
  flour, 680–684
    and bread, 760
    caloric value, 682
    composition, 681–683
    lipid content, 104
    minerals in, 682
    production, 680
    roasted, composition, 779
    shells, 51, 84
    uses, 683–684
    vitamins in, 682
  minerals in, 281, 284
  protein amino acids in, 619
Peanut butter, 760
Peanut oil, 90, 95, 104, 565, 571, 576, 579, 584, 586, 589, 760
  characteristics, 582
  composition, 581
Pear, 28, 776
  Bartlett, composition, 779
  composition, 779, 812
  dried, composition, 779
  flavoring, synthetic, 898
  fresh, carbohydrate content, 783
Pecan, 760
  composition, 288, 779, 812
  oil, 760
Pectin(s), 22, 813, 819–821
  commercial, 821
  in cucurbits, 730
  in fruits, 766, 767, 770, 774, 821
  structure, 820
  uses, 821
  in vegetables, 736, 821
Pectose, 820
Pellagra, 241, 664. See also *Niacin amide*.
Penicillin, 370
Penicillium, 719, 720, 721
Pentacetyl-α-D^{+}-glucose, 83
Pentacetyl-β-D^{+}-glucose, 83
Pentamethylenediamine, 143
Pentosan(s), 84, 616, 621, 638, 641, 642, 732
Pentosan(s) (contd.)
  in coconut, 762
  in legumes, 743, 745
Pentose, 68, 84, 349, 819
  in coconut, 762
Pepper, black, 890
    composition, 877, 878
  Cayenne, 891
    composition, 877, 878
  long, 891
  red, 891
  Singapore, 891
  Tellicherry, 891
  white, 891
    composition, 877, 878
Peppers, composition, 758
  garden, 732–733
  green, 733, 758
    carbohydrate content, 783
  red, 733, 758
Pepsin, 117, 123, 136, 162, 185, 194, 197, 198, 200, 208, 209, 210, 212, 214, 215, 333, 334, 335, 336, 337, 340, 468, 795
Pepsinase, 211
Pepsinogen, 215, 335
Peptidase(s), 136, 211, 212, 214, 336, 339–341, 342, 343, 344, 796
  gastrointestinal, significance, 343–344
  intestinal, 352
Peptide(s), 113, 116, 135, 137, 138, 140, 141, 153, 155, 173, 212, 336, 340, 341, 343, 344, 352, 360
  bond, 212, 336, 340
  chains, 115, 147, 163–164, 165, 166, 182, 215, 336
  isomeric forms, 146
  linkage, 115, 135, 136, 146, 152, 153, 163
  properties, 146–148
  synthetic, 134–139, 212
    hydrolysis of, 336
Peptization, 36
Peptone, 113, 116, 135, 136, 198, 200, 336, 360, 468
Perch, yellow, 480, 500
Perilla oil, 91
Peristalsis, 337
Periwinkle, 246
Perosis, 288
Peroxidase, 177, 185, 199, 209, 219, 222, 528. See also under individual enzymes.
  in apple, 774
  in milk, 405, 408
  root, 194
  unit, 209
Persea americana, 776
Persian berry, 301, 324
Persian walnut. See *Walnut*.
Persimmon, carbohydrate content, 783
Persulfate, inhibiting action on enzymes, 623
Pet foods, 469
Petroselinum
  crispum latifolium, 890
  hortense, 890
  sativum, 890
Petunia hybrida, 318
Petunidin, 318
*p*H, 26–35

pH (contd.)
  of acids, 28
  application of, 34, 35
  of bases, 28
  of cake flour, 702
  for cakes, optimum, 703–704
  of cherries, 769
  determination, 30–33
    colorimetric, 30–31
    electrometric, 31–32
    electrometric titration, 33
  of fish, 493
  isoelectric point and, 33
  and jelly strength, 33
  of milk, 402–403
  and mold growth, 721
  and staling
  of various common substances, 27, 28
Phaselin, 743
Phaseolin, 111, 179, 619, 743
Phaseolus
  limensis, 744
  lunatus, 744
  vulgaris, 742
Phellandrene, 886
Phenol, 144, 145
  in large intestine, 354
Phenolphthalein, 27
Phenylacetic acid, 142
Phenylacetylglutamine, 142
Phenylalanine, 106, 117, 120, 123, 124, 125, 127, 128, 130, 131, 132, 133, 135, 142, 143, 154, 180, 182, 257, 258, 336, 340, 352, 527, 618, 621, 743, 747
β-Phenylethylamine, 143
Phenylvaleric acid, 106
Pheohemin, 216, 220, 222
  protein, 205
Phlobaphene, 863
Phlorizin, 142, 349, 350, 353
Phoenix dactylifera, 779
Phosphatase, in milk, 405, 408
  unit, 208
Phosphate(s), 30, 198, 342. See also specific compounds.
  in baking, 687, 691
  in berries, 763, 765
  in English walnuts, 761
  in fruit, 767, 768, 769, 770, 773, 775
  in legumes, 743, 745, 746, 747
  in pumpkin, 730
  in truffles, 758
  in vegetables, 738, 751, 756
Phosphatides, 88, 621
Phosphocreatine, 801
Phosphodihydroxyacetone, 801, 802
Phosphogluconic acid, 217
Phosphogluconic dehydrogenase, 218
Phosphoglyceraldehyde, 218
Phosphoglyceric acid, 218
α-Phosphoglyceric acid, 800, 801, 802, 806
β-Phosphoglyceric acid, 800, 801, 806
Phosphoglycerol, 801, 802
Phospholipid(s), 88, 100–103, 105, 177, 350, 351, 487
Phosphoproteins, 112, 167, 174–175
Phosphopyruvic acid, 800, 801, 802, 806
Phosphoric acid, 28
Phosphorus, 270, 276–280, 286, 295, 351, 464
  in blood, 277
  in bone, 276–277
  in cabbage, 737
  calcium ratio, 710
  in cereal grains, 626, 627, 630, 637, 646
  feed variations, 279
  in fish, 486
  in various flours, 682
  food variations, 279
  in milk, 405, 407
  and rickets, 277–278
  in urinary calculi, 278
Phosphorylase, 204
Phosphorylation, 105, 204, 350, 353
Phrenasin, 89, 103
Physetoleic acid, 90
Phytin, 198, 280, 627
Phytosterol, 98, 100, 745
Pickle, 28, 729
Picramnia seed fats, 91
Picric acid, 303
Picrocrocin, 892
Pies, carbohydrate content, 783
  staleness, 706
Pig, breeds, 438
  crops, 436
Pigments. See *Coloring matters.*
Pike, blue, 480, 500
  yellow, 480, 500
Pilchard, 478, 479, 483, 484, 486, 489, 492
Pimenta officinalis, 880
Pimento, 28, 880, 892
Pimiento, 892
Pimpinella anisum, 880
Pineal gland, 269
Pineapple, 780
  composition, 779, 812
  flavoring, synthetic, 898
  fresh, carbohydrate content, 783
  juice, raw, 468
Pine disease, 293
Pinenut, 760
  composition, 779
Pink. See *Dutch pink.*
Pinus, 760
Piper
  longum, 891
  nigrum, 890
Piperidine, 891
Piperine, 891
Pistachio, 762
  composition, 779
  shelled, carbohydrate content, 783
Pistacia vera, 762
Pisum sativum, 746
Pitocin, 261
Pitressin, 261
Pituitary gland, 261
  hormones, 261–262
  substance, anterior, 468

Plants, 110
  mineral metabolism of, 276
  poisonous, 381
  proteins, 619
Plasticity, 11, 19, 20, 40, 41
  determinations, 580
  of milk, 399
  of shortening, 587
Plasticizer, 79
Plastometer, 21
  capillary-tube, 21
  power-consumption, 21
Platichtys stellatus, 488
Platyhelminths, 391
Pleuronectidae species, 486
Plum, 28, 300, 771–772
  carbohydrate content, 783
  composition, 779
Plymouth rocks, 524. See also *Chicken.*
  composition, 525
Pneumatophorus diego, 489
Poisons. See *Food.*
Polarimeter, 17
Polenske value, 573, 583
Pollachius vireus, 488
Pollock, 475, 483, 488, 491, 500, 513
Polygalacturonic acids, 820
Polygonaceae, 592
Polygonoideae, 592
Polymannuronic acids, in agar-agar, 822
Polynucleotidase, 168, 795
Polypeptidase(s), 198, 211, 212
Polypeptide(s), 135, 136, 138, 141, 163, 173, 343, 352, 360
  chains, 164, 165, 166, 182
  properties, 146–148
  synthesis, 134–139
Polyphenoloxidases, 217, 222
Polysaccharide(s), 41, 68, 78, 82, 150, 173, 819
  bacterial, 174
  gastric, 174
  muco-, 173, 174, 329
  reactions of, 85–87
  in vegetables, 734, 735, 750, 751
Pomegranate, 781
  carbohydrate content, 783
  composition, 779
Pomes, 774–776
Ponceau 2G, 302
Ponceau 3R, 302, 303, 304, 307
  reactions, 304
  spot tests, 307
Ponceau SX, 303, 307
  reactions, 307
  spot tests, 307
Popcorn, 592, 600, 615, 616, 726
Poppyseed oil, 91, 581, 584
  characteristics, 582
  composition, 581
Porgy, 475
Pork, 391, 439
  canned, federally inspected, 440
  classes and grades, 444
  composition, 445
Pork (contd.)
  consumption, 439, 440
  fat, rendered, 467
    used for margarine, 588
  production, 439
  sausage, carbohydrate content, 783
  tapeworm in, 391
  and trichinosis, 391, 463
Porphyrin, 175
  complex, 176, 177
  nuclei, 216, 345
  residue, 217
Porpoise, body oil, 95
  oil, 90
Potash, in almonds, 759
  in berries, 763, 764, 765
  in fruits, 766, 767, 768, 769, 770, 771, 772, 773, 775,776, 780
  in lentils, 743, 745, 746, 747
  in truffles, 758
  in vegetables, 732, 738, 748, 751, 756
Potassium, 270, 272, 281–282, 295, 485
  in cereals, 626, 627
  in milk, 407
Potassium compounds. See also *Potash.*
  bitartrate, 717
  bromate, 652, 658, 696
  iodate, 652
Potato, 217, 752–756
  amino acids in, 619
  bacillus. See *Bacillus mesentericus.*
  carbon dioxide formed by, 753–756
  chips, 726
    carbohydrate content, 783
    composition, 758
  composition, 288, 753
  frozen, 10
  raw, carbohydrate content, 783
  respiration, 753–754
  starch, 815
  sugar-starch exchange in, 754–755
  sweet. See *Sweet potato.*
  tuberin, 619, 756
Poultry, 522–540
  bleeding and braining, 528
  bloom, 537
  chicken. See *Chicken.*
  cooling of, 530–532
  decomposition of, 532–533
  dressing of, 528–540
    market grades, 526
  evisceration of, 538–540
    New York City regulations, 539–540
  fat, 527
    acidity, 533
  flesh, 524
    amino acids in, 527
    composition, 525
    composition before and after feeding, 526
    protein nitrogen in, 527
    weight, distribution, 526
  freezer burn, 537
  freezing of, 533–538
  geographical distribution, 522–523

Poultry (*contd.*)
  picking of, 528–530
  turkeys, 522, 523–524
Pregnanediol, 265
Pregnenin-on-3-ol-17, 265
Primioa lepalifero, 122
Progesterone, 263–265
Prolactin, 262
Prolamin, 112, 128, 617, 618, 620, 647
Prolan, 226
Prolan A, 261
Prolan B, 261
Proline, 112, 117, 121, 123, 124, 125, 128, 131, 132, 133, 135, 139, 140, 141, 142, 143, 152, 154, 155, 156, 164, 180, 527, 618, 743, 745, 747
Prolysine, 123
Pro-oxidants, 568
Propionates, and mold growth, 722
  and rope infection, 717, 718
Propionic acid, and mold growth, 108, 712, 721
  salts of, and mold growth, 721
Prosthetic group, 166, 203, 214, 215, 216, 219, 220, 221, 222, 223, 226
Protachysterol, 248
Protamin, 112, 114, 128
  -zinc-insulin, 268
Protamin nucleus hypothesis, 165
Proteans, 113
Protease(s), 211, 213, 695–696, 713, 758
  in baking, 695–697
  control by oxidation, 696
  effect on gluten proteins, 695–696
  effect of milk, 696–697
Proteinase(s), 203, 207, 209, 212, 211, 632
  unit, 209
Protein(s), 10, 22, 30, 33, 38, 39, 40, 41, 42, 48, 109, 110–187, 195, 197, 198, 207, 212, 213, 224, 273, 336, 341, 358, 464. See also *Virus*.
  alcohol-soluble, 112
  amino acids in, 117, 126–127, 618–619
  analytical procedures, 151–157
    color reactions, 152, 153
    quantitative methods, 153–157
  in animal tissues, 126, 619
  in bakery products, 710
  of bean, 743
    oil seeds, biological value, 680
  in breakfast foods, 678–679
  cereal, 178, 618–619
  in cereal feeds, 631, 632
  in cereal grains and cereal products, 617–761, 629, 630, 631, 632, 635, 641, 646, 655
  classification of, 111–114
  coagulated, 113
  in coffee, 844
  conjugated, 112, 166–167, 185, 216–219, 617
    chromoproteins, 112, 167, 175–177
    glycoproteins, 112, 114, 167, 172–174, 329
    nucleoproteins, 112, 167–172, 175, 219, 617, 620
    phosphoproteins, 112, 167, 174–175
  copper, 217
  crystalline, 114, 165, 214
  decomposition in foods, 359–360, 361
  denaturation of, by cooking, 462
Protein(s) (*contd.*)
  derived, 112–113
  digestion of, 337, 352–353
    products of, absorption, 352–353
  in egg, 39, 126, 181, 283, 619. See also ***Vitellin***.
  fibrous, 165
  in fish, 483–485, 619
  flavo-, 170, 216, 217, 219, 221, 222, 223
  in various flours, 682
  in fruits and fruit juices, 180, 770, 773, 778–779
  in gelatin, 619
  gluten, 694, 695, 702
  hemin, 217, 221, 222
  hydrolysis of, 115–119, 200, 348, 623
  isolation of, 114–115, 182
  lipo-, 177
  in meat, 126, 445, 446
  meta-, 113
  metallo-, 216, 222
  in milk and milk products, 404, 405, 406, 409, 411, 412, 413, 423, 425, 428, 430, 431, 433, 619
  molecular weights of, 157–163
    from chemical composition, 157, 158
    from sedimentation velocity, 161
    by ultracentrifuge, 160
  in mushrooms, 757
  nucleates, 172
  in nutrition, 177–182
  in nuts, 759, 761, 778–779
  plant, 618–619
  in poultry, 525–526, 619
  pyridino-, 216, 218, 221, 223
  relationships with carbohydrates and fats, 806–809
  sclero-, 112
  simple, 111–112, 185, 214–216
  spinal cord, 121
  structure, 163–167, 183
  in vegetables, 128, 180, 742, 753, 757–758
  in wheat flour and gas retention, 694
  zinc, 222
Proteolysis, 360
  and food spoilage, 358
Proteose, 113, 116, 123, 135, 336, 617
Prothrombin, 177
Protoctine, 123
Protoferriheme, 219
Protoferriheme IX, 220, 222
Protohemin, 216, 221
Protopectin, 820
  in fruits, 774
Protoplasm, 110
Protozoa and food spoilage, 391
Provitamins A, 230–233
  conversion to vitamin A, 232–233
Provitamins D, 245–247
  conversion to vitamins D, 247–248
Prune, 771–772
  carbohydrate content, 783
Prunus
  amygdalus, 759
  armeniaca, 769
  avium, 769
  cerasus, 770

Prunus (*contd.*)
  domestica, 771
  persica, 770
Prussian blue, 300
Pseudomonas, 390, 543
  pyocyaneus, 361
Pseudopleuronectes dignabilis, 488
Pterocarpus santalinus, 318
Ptomaine, 143, 354, 360
  poisoning, 380
Ptyalin, 329, 790, 795
Pullets, 523. See also *Chicken*.
Pumpkin, 28, 729–730, 731
  ash, 730
  canned, composition, 758
  carbohydrate content, 783
  composition, 758
  ripening, chemical changes during, 730
  total solids, 730
Punica granatum, 781
Purine, 168, 172, 175
  bases, 167
  nucleoside, 168
Purpurin, 323
Purpurogallin, 209
  number, 209
Putrefaction, 142, 143–146, 354, 380
Putrescine, 143, 146, 354, 360
Pycnometer, 11
Pyocyanine, 185, 204, 361, 370
Pyranoid ring structure, 61, 62
Pyridine-3-carboxylic acid, 241
Pyridine nucleotide, 170, 171
  oxidases, 171
Pyridinoprotein, 216, 218, 221, 223
Pyridoxine, 187, 223, 230, 239–240
  in cereal grains and products, 626, 643, 656
  in fish, 491
  in milk, 405, 407
Pyrimidine, 168, 172, 175
  bases, 167
  nucleoside, 168
Pyrimidine-thiazole phosphoric acid, 220, 221
Pyrogallol, 94
Pyrrole, 165, 176
  hypothesis, 165
α-Pyrrolidine carboxylic acid, 121
Pyrus
  communis, 776
  malus, 774
Pyruvic acid, 108, 143, 188, 207, 219, 223, 800, 801, 802, 806
  dehydrogenase, 188, 219, 223, 224
Pyruvic aldehyde, 803, 807, 808, 809

Q

Quebracho, 318
  bark, 318
Quebracho colorado 318
Quercetin, 318, 324
Quercitron, 324
  bark, 318
Quercus tinctoria, 318, 324
Quince, 776
  composition, 779
Quince seed gum, 819
Quinhydrone, 33
γ-Quinoline pyruvic acid, 207
Quinotannin, 318

R

Rabbit, 75
Racemization, 133, 134
Radicula armoracia, 887
Radish, 749
  carbohydrate content, 783
  composition, 288, 758
Raffinose, 51, 78, 621, 788–789, 791
  in beet, 748
  in coconut, 762
  in soybean, 745
Raisin, carbohydrate content, 783
  composition, 812
  iron content, 286
Raja
  binoculata, 489
  stellulata, 489
Rancidity, 94, 95, 362, 378, 567–568
  in fish, 494
  test for, 577
Rape oil. See *Rapeseed oil*.
Rapeseed oil, 90, 565, 567, 571, 584
  composition, 581
Raphanus sativus, 749
Rapic acid, 90
Raspberry, 763–764
  black, composition, 779
  carbohydrate content, 783
  red, composition, 779
Rectum, 328, 354
Red, fast, E, 305
Reductase, in chicken fat, 528. See also under individual enzyme.
Refractive index, 10, 13–17
  definition, 13
  of fats and oils, 574, 581, 582, 584
    hydrogenated oils, 583
  variation with temperature, 14, 583
  variation with wave length, 13
Refractometer, 14–16
  Abbé, 14
  compensation, 14
  Goerz, 15
  immersion, 15
  special, 15
  Zeiss, 15
Refrigeration, 7, 375, 391, 451–452, 530
Reichert-Meissl number, 96, 473, 583
Renin, 269
Rennin, 334, 335, 468, 795
Resorcine yellow, 303
Respiration, of berries, 763, 764, 765
  of cereal grains, 612–614
  of fruits, 766, 770, 771, 773, 774, 777, 780
  of pumpkin, 730
  of vegetables, 733, 736, 749, 753–754

Rhamnazin, 324
Rhamnetin, 324
Rhamnoheptose, 50
Rhamnose, 50, 78, 350
Rhamnus amygdalinus, 324
Rheinhartius hippoglossoides, 488
Rheum rhaponticum, 740
Rhizopus, 719, 720
Rhode Island red, 524. See also *Chicken.*
Rhubarb, 28, 381, 740
  canned, composition, 758
  carbohydrate content, 783
  composition, 758
  petioles, composition of, 740
Rhus coriaria, 318
Ribes
  hirtellum, 765
  rubrum, 318, 764
Riboflavin, 221, 224, 226, 230, 237–239, 298, 353, 624–625
  in bread, 711
  in cereal foods, 630
  in cereal grains and products, 624–625, 642, 643, 644, 646, 656, 657
  and enrichment of flour, 662–667
  in fish, 491, 492
  in various flours, 682
  in milk, 405, 407, 408
Riboflavinphosphate, 170, 171, 217, 223
Riboflavinphosphoric acid, 220
  -adenine-dinucleotide, 238
Ribonuclease, 162
D^--Ribose, 50, 68, 70, 167, 221, 236
L^+-Ribose, 70
Rice, 591, 595, 610, 639–646
  amino acids in, 618
  biological value, 644–645
  botanical classification, 592
  bran, composition, 632, 641
  bran oil, 581, 584
    characteristics, 582
    composition, 581
  breakfast foods from, 678
  brewers', 640
    composition, 631, 641
  brown, 615, 624, 625, 639, 640, 644, 645
    composition, 629, 631, 641
    mineral content, 630, 644
    vitamin content, 630
  by-products, composition, 641
  by-products, vitamins in, 643
  carbohydrate content, 783
  classes, 610, 641
  composition, 615, 616, 641
  cultivated, 639–646
    composition, 641
  flakes, composition, 629
  flour, composition, 629
  germ oil, unsaponifiable fraction, 622
  globulin, 618
  glutelin, 618, 620, 644
  head, composition, 641
  lowland, 595
  Malekized, 643, 644
Rice (contd.)
  milled, B complex retention in, 642–643, 644
    utilization of, 645
  mineral constituents, 626, 627, 644, 646
  oryzenin, 620, 644
  polish, composition, 632
  polished, 639, 640
    composition, 631
  production, 593, 595–596
  puffed, composition, 629
  respiration of, 614
  rough, 610, 615, 616, 624, 625, 627, 639, 645
    composition, 615, 641
  types, 639–641
  upland, 595
  utilization of, 639, 645
  vitamins in, 624, 625, 642–643, 644, 646
  white, 640
    composition, 629
    mineral content, 630, 644
    vitamin content, 630
  wild, 645–646
    composition, 629, 646
Ricinoleic acid, 90, 97
Rickets, 249, 277. See also *Vitamin D.*
Rigor mortis, 447
  in fish, 493
Ringer-Locke solution, 274
Ripening. See *Fruits, ripening*, and under individual fruit or vegetable.
Roaster, 525, 532. See also *Chicken.*
Robison-Embden ester, 799
Rocella tinctoria, 319, 323
Rockfish, 479, 488, 500
Rodents, 390
Rolls, 688. See also under *Flour.*
  basic ingredients, 689
  composition, 710
Root(s), 51, 199, 748–751
  regarded as vegetable, 748–751
    beet, 748
    carrot, 748–749
    parsnip, 749
    radish, 749
    rutabaga, 749
    sweet potato, 749–751
    turnip, 751
Rope, 713–719, 720, 721, 722
  detection of, 716
  inhibition of, 716–718
  nature of, 713–714
  prevention of, 718
  sources of, 714–716
  spores, 715–716, 718
Rosanoff charts, 69, 70
  notation, 73
Rosefish, 475, 488, 500
Rosenheim reaction, 99
Roughage, 354
R salt, 304, 305
Rubia tinctoria, 319, 323
Rubus
  idaeus, 763
  vitifolius, 764

Rutabaga, 749
  composition, 758
Rye, 179, 591, 596, 610–612, 646–649. See also *Bread*.
  amino acids in, 618
  α-amylase in, 648
  amylase activity and baking quality, 647–648
  botanical classification, 592
  bran, 648
  breakfast foods from, 678
  carbohydrate gum, 647
  composition, 615
  definitions, 648–649
  feed, 648
    composition, 632
  flour, composition 629, 632, 647
    middlings, composition, 632
  gliadin, 618, 647
  glutelin, 618
  middlings, 648, 649
    composition, 632
  mill products, composition, 647
  mineral constituents, 627
  production, 593, 596
  proteins, 647
  red dog, 649
  respiration of, 614
  screenings, composition, 632
  standards, 648–649
    U. S. grain, 612
  utilization, 646
  vitamins in, 624, 625

S

Sablefish, 479, 510, 513
Saccharase, 197, 198, 201, 202
  activity curve, 201
  unit, 208
  in yeast, 195, 197, 200
Saccharic acids, 80
Saccharide(s), 49–87, 792. See also *Carbohydrate*.
Saccharimeter, 19
Saccharin, 68, 818
Saccharolactone, 81
Saccharomyces, 830
  cerevisiae, 712
  ellipsoideus, 712
Safflower, 318, 324
  oil, 581
    characteristics, 582
    composition, 581
Saffron, 325, 892
Safranine, 303
Sage, 892–893
Sakaguchi reaction, 152
Salad dressing, 43, 47, 900–901
Saliva, 27, 28, 112, 198, 795
  in digestion, 327, 328, 329–330, 795
  human, 28, 329
  secretion of, 330
Salkowski reaction, 99
Salmine, 112, 117, 128, 619
Salmo gairdneri, 488
Salmon, 28, 478, 492, 504–507, 513
  blueback, 479, 505
  canned, 481, 483, 486, 504–507
  chinook, 479, 482, 486, 505
  chum, 479, 480, 483, 484, 486, 488, 505
  coho, 479, 483, 486, 505
  cured, 512
  fresh, 104, 505
  humpback, 479, 505
  iodine in, 288
  keta, 479, 505
  king, 479, 480, 483, 484, 488, 505
  kippered, 510
  pickled, 513
  pink, 479, 480, 483, 484, 486, 488, 505
  red, 479, 480, 482, 486, 491, 505
  silver, 479, 480, 484, 488, 505
  smoked, 510
  sockeye, 483, 484, 488, 505
  steelhead, 488
Salmonella, 465
  in food poisoning, 388–389
Salt, 12, 281, 282, 373
  in baking, 687, 689, 697
  in cake, 706
  in curing, 368, 453
  and food spoilage, 368–369
Salts, absorption of, 348–349
Salvia officinalis, 892
Sanderswood, 318, 325
Sanitation and food preservation, 378–379
Santalin, 325
Sap, 51
Saponification, 93
  number, 93, 572, 581, 582, 584
Saponin, in soybean, 745
Sarda chilensis, 489
Sardine, 479, 483, 491
  characteristics, 582
  composition, 581
  oil, 91, 581, 584
Sardinia caerulea, 486, 489
Sardinops caerulea, 489
Satureia hortensis, 893
Sauerkraut, 28, 358
Sauger, 480, 500
Sausage, 460–461
  Bologna, carbohydrate content, 783
  casings, 468
  pork, carbohydrate content, 783
  summer, 373
Savory, 893
Saybolt viscosimeter, 21
Scallop, 475, 516
  muscle protein, amino acids in, 619
  sea, 476
Schaal method, 578
Schaeffer's acid, 313
Schardinger enzyme, 199, 210
Schütz rule, 200
Scleroproteins, 112
Scoliodon longuris, 489
Scomber scombrus, 486, 489
Scombrine, 112

Scombrone, 112
Scup, 475, 476
Scurvy, 245. See also *Vitamin C.*
Scutellum, 600, 601, 602, 622
Seal oil, 90, 95
Sebastes marinus, 488
Sebastodes
  mystinus, 488
  paucispinis, 488
  ruberrimus, 488
Secale cereale, 592
Secretagogues, 332, 344
Secretin, 269, 338
Sedimentation velocity, 161
Selachyl alcohol, 487
Selenium, 270, 271, 292–293, 626
Semolina, 606, 670–673
  composition, 672
  definition, 673
  production, 649
  standards, 673
  use in macaroni products, 674
Seralbumin, 126
Serine, 117, 119, 124, 130, 132, 133, 142, 154, 180, 618, 743, 747
Seriola dorsalis, 489
Serratia marcescens, 361, 719
Serum, 126, 173
  albumin, 111, 113, 117, 277
    horse, 162
  globulin, 111, 126, 117, 141, 150
  mucoid, 173, 174
Sesame oil, 95, 571, 576, 584, 586
  antioxidants in, 584
  characteristics, 582
  composition, 581
Shad, 476, 477, 484, 510
  roe, carbohydrate content, 783
Shark, 479, 489
Sheep, 437, 441
  breeds, 438
  casings, 468
  classes and grades, 444
Sheepshead, 480
Shellfish, 516–519
  of Chesapeake Bay States, 477
  essential amino acids in, 484
  of Middle Atlantic States, 476
  of New England States, 475
  production, 516
Sherbet, 427
Shortening, 467, 579, 586, 661, 710, 762
  agents, 47, 586–588
    characteristics, 587
    free fatty acids in, 587
    iodine value, 587
    plasticity of, 580, 586, 587
    stability of, 587
  in baking, 687, 688, 689, 697, 703, 705
  in cake, 703, 705, 706
  hydrogenated, 703
  superglycerinated, 580
Shrimp, 28, 478, 480, 484, 486, 516, 519
  canned, 481
Shrimp (contd.)
  iodine in, 288
  muscle protein, amino acids in, 619
Silicon, 270, 292, 485, 626
Sinalbin, 888
Sinapine hydrogen sulfate, 888
Sinapis alba, 888
Sinigrin, 887, 888
Sirup, 22, 53, 784. See also *Corn Sirup.*
  in cake making, 705
  cane, 817
  composition, 818–819
  golden, 817, 818
  maple, 817, 818
  molasses, 817
    composition, 812, 818
  refiners', 817
  sorgo, 817, 818
  from sugar cane, 817, 818
  top, 817
Sitosterol, 100, 485, 573, 622
γ-Sitosterol, 99
Skate, 489
Skatole, 143, 145, 354, 360
Skin, 110, 112. See also *Hide.*
Skipjack, 482
Slipped tendon, 288
Smelt, 479
Smoke point, 580, 583
Snail, 112
Snakeroot poisoning, 381
Snapper, red, 478, 484, 486, 500
Soap, 27, 36, 42, 44, 93, 97, 105
Soda, in celery, 730
  in pumpkin, 730
Sodium, 270, 272, 295, 626, 627
  and chlorine, 281–283
  -potassium ratio, 281–282
Sodium compounds. See also *Soda.*
  acetate, 30
  bicarbonate, 27, 28, 281
    in flour, 660
  carbonate, 28
  chloride. See *Salt.*
  diacetate, 717
  fluoride, 381
  glycocholate, 345
  hydroxide, 25, 26, 27, 28
  lactate, 805
  metasilicate, 28
  nitrate, in curing, 453
  nitrite, in curing, 453, 455
  propionate, 717, 718
  taurocholate, 345
Soil factors and nutritional quality, 297
Sol, 35, 37, 38, 39–40. See also *Colloid.*
Solanum
  melongena, 732
  tuberosum, 752–756
Sole, 488
Solidification point, 92
Somniosus microcephalus, 489
Sørensen method, 153
Sorbitol, 65, 79

- *D*-Sorbitol, 60
- Sorbose, 65, 349, 350
- Sorghum, 817
  - cane, 51
  - grain, 591
    - amino acids in, 618
    - botanical classification, 592
    - production, 593, 597
    - prolamin in, 618
    - respiration of, 614
    - thiamine in, 624
    - varieties, 597
- Sorgo, 812, 817, 818
- Sours, 698
- *Soya max*, 745
- Soybean, 99, 127, 179, 198, 272, 279, 745
  - albumin in, 745
  - calcium content, 279
  - canned, composition, 758
  - carbohydrates of, 745
  - flour, 680–684, 745
    - caloric value, 682
    - composition, 681, 683
    - glycinin in, 681, 684
    - macaroni products, 676
    - minerals in, 682
    - production, 681
    - uses, 683–684
    - vitamins in, 682
  - fresh, composition, 745
  - glycinin, amino acids in, 619
  - meal, 122, 745
  - oil, 95, 565, 566, 567, 571, 576, 577, 579, 584, 586, 589, 745
    - characteristics, 582
    - composition, 581, 745
    - hydrogenated, 584
  - proteins in, 745
- Soylegumelin, 745
- Spaghetti, 673, 674, 675
- Specific gravity, 11–12. See also *Density*.
  - of milk, 398
  - of peas, 746
  - and starch content of potato, 752
- Specific heat, 400
  - of milk, 400–401
  - of oils, 583
- Sperm, blubber oil, 98
  - head oil, 98
  - oil, 90
  - whale oil, 98
- Spermaceti, 90
- Sphingomyelin, 90, 100, 102, 105
- Sphingosine, 100, 102, 103
- *Sphyraena argentea*, 489
- *Sphyrna zygaena*, 489
- Spices, 3, 876–893. See also under individual spices.
  - adulteration of, 879
  - chemical composition, 877–878
- Spinach, 28, 280, 741
  - canned, composition, 286, 758
  - composition, 286, 758
- *Spinach* (*contd.*)
  - protein, 742
  - trimmed, chemical changes in, 741
- *Spinacia oleracea*, 741
- Spinacin, 742
- Spinal cord, 100
- Spinal fluid, human, 28
- Spleen, 51, 126, 167, 169, 195, 198
- *Squalus*
  - *acanthias*, 489
  - *suckleyi*, 489
- Squash, 28
  - carbohydrate content, 783
  - summer, 731
    - composition, 758
  - winter, 730–731
    - composition, 758
    - ripening, chemical changes during, 730–731
- Squashseed, 179
- Squeteague, 476, 477, 478, 483, 484, 500
- Stabilizers, 42, 819
- Stachyose, 788. See Tetrasaccharide.
- Stacker burn, 509
- Stag, 442
- Stalagmometer, 23
- Staleness, 706
  - development of, 706
    - in cake, 709
  - methods of measurement of, 707, 708
  - *p*H and, 709
  - physical changes caused by, 707
  - prevention of, 708
    - by freezing, 706
- Staphylococci, enterotoxin of, 386
  - food poisoning, 385–388, 389, 390
  - laboratory diagnosis of, 387
- *Staphyloccus*, 361, 364
  - *albus*, 465
  - *aureus*, 465
- Star aniseed, 880–881
- Starch, 9, 22, 40, 44, 51, 52, 53, 63, 68, 78, 79, 85, 87, 194, 198, 200, 204, 336, 339, 341, 615, 621, 622, 691, 692, 702, 714, 786, 787, 790, 792
  - in cacao, 830
  - cassava, 595
  - corn, 73–74, 816
  - in cucurbits, 730
  - esters of, 86
  - ethers of, butyl, 86
  - in fruits, 773, 775
  - hydrolysis of, 329, 622, 795
  - in legumes, 745, 746
  - molecule, 63
  - in nuts, 762, 779
  - phosphorylase, 214
  - propyl ethers of, 86
  - soluble, 692
  - and specific gravity, 752
  - and staleness, 706
  - structure of, 63, 787
  - and sugar exchange, 754–756
  - in vegetables, 733–735, 736, 749, 750, 752, 753, 757, 758

Starch (*contd.*)
  waxy, 595
  xanthates, 86
Starters, 698
Steapsin, 339, 796
Stearic acid, 90, 92, 96, 102, 106, 108, 565, 581, 582, 737, 745, 760, 762, 808
Stearin, 92, 93, 95
Stearo-diolein, 92, 95
β-Stearo-diolein, 92
Stearyl alcohol, 98
Steer, 442
Stems, vegetables which are. See *Leaves and stems.*
Stercobilin, 354
Stereoisomerism, 99
Stereolepsis gigas, 489
Steroid derivatives, 351
Sterol, 88, 98, 247, 264, 351, 487, 573, 621
  in cabbage, 738
  compounds, 98, 100, 267, 745
  derivatives, 262, 264
  ring structure, 99
Stigmasterol, 99
Stizolobin, 619
Stomach, 198
  contents, human, 27, 28
  diagram of, 331
  digestion, function in, 330–337
  enzymes in, 334–336
  gastric juice, 185, 333–336
  secretion by, 331, 332, 333
Storage, 7, 10
  of cereal grains, 612–614, 735
  and food spoilage, 364–365
  of fruits, 768, 775
  of vegetables, 738, 750, 754, 755
Straw, 51, 84
Strawberry, 28, 763
  carbohydrate content, 783
  composition, 778
  flavoring, synthetic, 898
Strengtheners, 661
Streptococci, 390
Streptococcus viridans, 465
Strings and ligatures, 472
Strontium, 485
Strychnine, 381
Sturgeon, 510
  sperm, 112
Sturine, 112
Succinic acid, 143, 204
  in blackberry, 764
  in broccoli, 732
  in synthetic flavors, 897
Succinic dehydrogenase, 194, 199, 207
Succotash, 744
  canned, carbohydrate content, 783
Succus entericus, 341, 345, 349
  digestive properties, 342
Suckers, 480
Sucrase, 85, 342, 690
Sucrol, 818
Sucrose, 11, 17, 34, 51, 52, 61, 64, 68, 74, 78, 81, 85, 192, 197, 198, 200, 201, 208, 349, 600, 621, 690, 782, 789, 790–791, 792, 818
  in candy, 813, 814, 815
  in coconut, 762
  in cucurbits, 730
  in fruits, 766, 770, 771, 772, 773, 774, 775, 776, 780
  in legumes, 745, 746, 747
  in pecans, 760
  in vegetables, 733, 736, 738, 741, 748, 749, 750, 755, 756
Sucrose octaacetate, 52
Sudan I, 303
Sugar(s) 12, 14, 16, 19, 25, 47, 52, 337
  absorption, relative rates of, 350
  acids, 79
  in baking, 687, 689, 692, 709
  beet, 6, 25, 51, 74, 785, 813, 814
  brown, composition, 812
  in cake, 704, 705, 706
  in candy, 812, 813, 814
  cane, 51, 52, 74, 784, 813, 814
  in cereal grains, 600, 615, 621, 641, 655
  in chocolate and cocoa products, 814
  consumption, 815
  in cucurbits, 730, 731
  decolorization of solutions, 39
  digestion of, 795
  epimeric, 58, 61, 67
  and food spoilage, 369
  foods, 782–809
  in fruits, 740, 766, 768, 769, 771, 772, 773, 775, 776, 777, 778–779. See also under *reducing* below.
  interconversion of, 793–794
  invert, 813, 816
  isomeric, 59
  ketose, 60, 791
  in legumes, 743, 745, 746
  in meat curing, 453, 456
  nonreducing, in asparagus, 751
  in nuts, 778–779
  preparation of, 74
  reducing, in fruits, 768, 770, 771, 774, 775, 776
    reactions of, 78–85
    in vegetables, 734, 738, 750, 751, 755
  refined, 782
  refraction of, 15
  simple, 65
    anabolism of, 796–797
  sweetness of, 791, 818
  in vegetables, 733–735, 738, 741, 750, 753, 757–758. See also under *reducing* above.
o-Sulfbenzoic acid, 70
Sulfhemoglobin, 460
Sulfhydryl compounds, activating enzymes, 623
Sulfolipids, 89
Sulfomucins, 114
Sulfur, 158, 270, 272, 283–284, 295, 626, 627
  amino acids containing, 120, 121, 283
  in brain, muscle, blood, 284
  essential organic compounds containing, 256, 260
  in foods, 284

- *Sulfur* (*contd.*)
  - in proteins, 126–127
  - radioactive, 181–182
- Sulfuric acid, 25, 26, 28, 173
- Sullivan's reaction, 152
- Sumach, 318
- Sunflowerseed oil, 565, 566, 571, 576, 584, 586
  - characteristics, 582
  - composition, 581
- Sunset yellow FCF, 303, 313
  - reactions, 313
  - spot tests, 313
- Suprasterols I and II, 100, 248
- Surface tension, 11, 23–25, 33, 38, 45, 46, 402
  - factors affecting, 24
  - measurement of, 23, 24
- Sweetbreads, 465
- Sweet potato, 28, 749–751
  - canned, composition, 758
  - carbohydrate changes in, 749–750
  - carbohydrate content, 783
  - composition, 758
  - effect of cooking on, 750
  - effect of curing, 750
- Swift method, 578, 587
- Swine, 441
- Swiss chard. See *Chard.*
- Swordfish, 475, 489
- Syneresis, 40–41

T

- Tachysterol, 100, 248
- *Taenia*
  - *saginata*, 391
  - *solium*, 391
- Talc, 300
- Tallow, 467, 565, 566, 570, 571, 589
  - bayberry, 90
  - beef, 95, 467, 585
    - characteristics of, 582, 583
    - composition, 581
  - Borneo, 835
  - mutton, 467, 585
    - characteristics, 582
    - composition, 581
- Talose, 58
- D^+-Talose, 56, 58, 70
- α-*D*-Talose, 72
- L^--Talose, 56, 58, 70
- Tangerine, 767
  - composition, 779
- Tankage, 469
- Tannin, 35
  - in apple, 774
  - brown, 865, 869
  - in cassina, 874
  - red, 863, 865, 866, 869
  - substances in cacao, 831
  - in tea, 863, 865, 870, 872
- Tapeworm, 391
- Tapioca, 595
- Tariric acid, 91
- Tarragon, 893
- Tartaric acid, 84
  - in grapes, 772, 773
  - in synthetic flavors, 897
- Tartrazine, 303, 305, 312, 313
  - reactions, 312
  - spot tests, 312
- Taurine, 144, 146, 283, 345
- Tea, 784, 856–875
  - adulteration of, 874
  - black, 861–868
  - botany of, 859–860
  - chemical aspects, 871–873
  - China, 872
  - classification, 861
  - consumption, 859
  - cultivation, 860
  - extracts, 52
  - fermented, 861
    - trade characteristics, 870
  - Formosa, 872
  - green, 861–868
  - India, 872
  - Japan, 872
  - Java, 872
  - manufacture of, 861–869
  - Oolong, 861
    - manufacture of, 869
  - *p*H, 863
  - production, 857
    - producing areas, 858
  - semifermented, 861
    - trade characteristics, 871
  - soluble solids in, 872
  - substitutes, 873–875
  - tannin, 863, 865, 866
  - trade characteristics, 869–871
  - types, 861
  - unfermented, 861
    - trade characteristics, 870
- Teaseed oil, 576, 584
  - characteristics of, 582
  - composition, 581
- Teeth, 277
- Tellurium, 278
- Tenderizing, of cake, 705
  - of flours, 661
  - of meat casings, 468
- Tendomucoid, 112, 173
- Tendon, 112, 173
  - slipped, 288
- Terpineol, 882
- Testosterone, 262, 263
- Tetany, 249, 277
- Tetramethylenediamine, 143
- 2,3,4,6-Tetramethylglucose, 62
- Tetranucleotide, 167
- Tetrapeptide, 147, 148, 344
- Tetrasaccharide, 788
- Tetrose, 65, 67, 73, 76
- *Thea japonica*, 859
- *Thea sinensis*, 859
- *Theobroma cacao*, 824, 828, 829
- Theobromine, 322, 831, 834, 835
- *Theragra chaleogramma*, 488

Thiamine, 224, 230, 234–237, 297, 298, 464, 569. See also *Vitamin B complex.*
  in bakery products, 660, 661, 711, 712
  in cereal grains, and products, 624, 625, 630, 638, 642, 643, 644, 646, 656, 657
  enrichment of flour, 662, 667
  in fish, 491
  in various flours, 682
  in milk, 405, 407, 408
  in sweet corn, 736
  iaminepyrophosphate, 219, 221
  iaminomagnesium-protein, 219
  aminoproteins, 216, 219, 223
  ochrome, 235
  ocyanogen, 94
  alue, 572
  olhistidine, 122
  iopropionic acid, 144
hreonine, 116, 120, 126, 127, 131, 132, 134, 143, 154. 179, 180, 257, 258, 618, 621
-Threonine, 117
Threose, 70
Thrombin, 177, 203
Thrombokinase, 203
hujone, 892
hunnus thynnus, 489
hyme, 893
Thymine, 168, 172
Thymol, 893
hymonucleic acid, 112
hymus, 112, 126, 269
  histone, 112, 177
  nucleic acid, 167, 168
hymus vulgaris L., 893
hyroglobulin, 148, 173, 174
hyroid, 112, 122, 128, 226, 267, 286, 287
  hormone. See *Thyroxine.*
hyronine, 149
hyroxine, 122, 128, 130, 131, 132, 133, 144, 145, 148–149, 267–268, 286, 352
Tiglic acid, 90
Timothy hay, phosphorus content, 279
Tin, 301, 485, 626
Titanium, 485, 626
Tocopherol, 250, 568, 569, 622
  allophanates, 250
  in hard wheat mill products, 658
omato, 28, 736–737
  canned, carbohydrate content of, 783
    composition, 758
  composition, 758
  green, composition, 758
Tonka bean, 896
Total solids, 16
  in cucurbits, 730
  in fruits, 740, 766, 768, 770, 772, 773, 775
  in milk and milk products, 404, 409, 419, 425, 430, 433
  in vegetables, 750, 751
ugheners, 661
  cake, 705
  sterol, 100, 248
  acanth, 43, 819, 822, 823
  sdehydroandrosterone, 262
Transphosphorylation, 204
Trehalosemonophosphoric acid, 800
Trematoda, 391
Triacontane, 774
Triakis semifasciata, 489
Tributyrin, 198
Tricalcium phosphate, 660
Trichinella spiralis, 391, 463
Trichinosis, 391, 463
Trichomona hominis, 391
Trifructosan, 647
Triglyceride, 339, 351, 564, 566
  mixed, 89, 91, 92, 564
  simple, 89, 564
2,3,4-Trihydroxybutanal, 65
1,3,4-Trihydroxybutanone, 65
Trihydroxyglutaric acid, 56
Triketohydrindene hydrate, 152
Trimethylamine, 494
  oxide in fish, 492, 494
2,3,6-Trimethylglucose, 62
Trimyristin, 890
Triolein, 564
Triose, 49, 73
Triosephosphate, 802, 806
Triosephosphoric enzyme, 218
Tripe, 126
Tripeptide, 135, 137, 138, 147
Triphenylchloromethane, 96
Triphosphopyridine, nucleotide, 221
Trisaccharide(s), 78, 82, 85, 87, 647, 788
  reactions, 85–87
Trisodium phosphate, 27, 28
Triticonucleic acid, 112
Triticum sativum, species, 592, 597
Tritylation, 95
Triundecylin, 107
Trout, gray sea, 476
  lake, 480, 484, 510
  sea, 477, 478, 483, 484, 500
Truffles, 758
Trypsin, 194, 195, 197, 198, 203, 209, 212, 214, 215 339, 340, 798
  complex, 185
  inhibitor-, 215
Trypsinogen, 203, 215, 339, 342
Tryptase, 211
Tryptophane, 117, 118, 121, 125, 126, 127, 128, 130, 131, 132, 133, 134, 143, 145, 152, 153, 154, 155, 158, 178, 179, 180, 209, 257, 258, 354, 484, 618, 620, 621, 645, 742, 743, 744, 745, 747
Tuberin, 619, 756
Tuber melanosporum, 758
Tullibee, 510
Tuna, 28, 478, 492, 507–508
  albacore, 479, 484, 489, 507
  bluefin, 479, 484, 489, 507
  bonita, 479, 484, 489
  canned, 507–508
  livers, 492
  skipjack, 479, 483, 484, 507
  striped, 489

- *Tuna* (*contd.*)
  - white meat, 507
  - yellowfin, 479, 489, 507
- Tung oil, 94, 95
- Turbot, 488
- Turkey, 522, 523–524
- Turmeric, 319, 325, 885
- Turnip, 28, 751
  - carbohydrate content, 783
  - composition, 758
- Tyramine, 143, 144, 145, 354
  - oxidase, 196
- Tyrosinase, 210, 217, 222
- Tyrosine, 117, 118, 120, 123, 125, 126, 127, 128, 130, 131, 132, 133, 134, 139, 142, 143, 144, 145, 149, 152, 153, 154, 155, 157, 158, 164, 175, 209, 215, 217, 222, 336, 340, 352, 354, 527, 618, 743
- *l*-Tyrosine, 128, 146
- Tyrosinosis, 143

U

- Ultracentrifuge, 160–162
- Ultrafiltration, 37
- Ultramarine blue, 300
- Ultraviolet light, 247
  - prevention of microbial growth by, 718, 723
- Uracil, 167, 172
- Urea, 142, 143, 154, 194, 198, 209
  - from carbon dioxide, 803
- Urease, 194, 198, 209, 214, 745
  - crystals, 210
  - unit, 209
- Uric acid, 143, 172
- Uricase, 745
- Urinary calculi, 278
- Urine, human, 28, 51
- Urobilin, 354
- Urocanic acid, 143
- Uronic acid, 65, 80, 174
  - in tragacanth, 822
  - units of gums, 819
- *Urtica dioica*, 321

V

- *Vaccinium*, 765
  - *macrocarpon*, 765
  - *myrtillus*, 318
- Vagotonine, 269
- Valine, 120, 124, 125, 126, 127, 132 133, 134, 154, 180, 257, 258, 618, 621, 743, 747
- Vanadium, 485
- Vanilla, 836
  - beans, 836
  - extract, 894–896
    - adulteration of, 896
    - composition, 895
  - fermentation of, 894
  - flavor, 836
- *Vanilla*
  - *fragrans*, 894
  - *planifolia*, 836, 894
  - *tahitensis*, 894
- Vanillin, 894, 895, 896
- *Vanillin* (*contd.*)
  - in clove, 884
  - content of vanilla extract, 895
- Vanillons, 836
- Van Slyke method, 153
- Van Wagenen chart, 548
- Vasopressin, 261
- Veal, 439
  - aging of, 448
  - chops, 104
  - classes and grades, 443
  - composition, 279, 446
  - consumption, 439, 440
  - production, 439
- Vegetable ivory, 68
- Vegetable(s), 3, 10, 105, 182, 252, 728–751. under specific vegetable.
  - bulbs, 728–729
    - garlic, 729
    - onion, 728–729
  - cucurbits, 729–731
    - cucumber, 729
    - pumpkin, 729–730
    - summer squash, 731
    - winter squash, 730–731
  - flowers, 731–732
    - artichoke, 731
    - broccoli, 731–732
    - cauliflower, 732
  - fruits, 732–737
    - eggplant, 732
    - garden pepper, 732–733
    - gumbo, 733
    - okra, 733
    - sweet corn, 733–736
    - tomato, 726–737
  - juices, 51
  - leaves and stems, 737–742
    - Brussels sprouts, 737
    - cabbage, 737–738
    - celery, 738
    - Chinese cabbage, 739
    - kohlrabi, 739
    - lettuce, 739
    - parsley, 739–740
    - rhubarb, 740
    - spinach, 741–742
    - Swiss chard, 742
  - legumes, 742–747
    - bean, 742–744
    - cowpea, 619, 783
    - lentil, 746
    - pea, 746–747
    - soybean, 745
  - roots, 748–751
    - beet, 748
    - carrot, 748–749
    - parsnip, 749
    - radish, 749
    - rutabaga, 749
    - sweet potato, 749–751
    - turnip, 751
  - shoots, 751–752
    - asparagus, 751–752

- *etable(s)* (*contd.*)
  - ubers, 752–756
    - Jerusalem artichoke, 756
    - potato, 752–756
- Vermicelli. See *Macaroni products.*
- Vetch, 179
- *Vicia faba*, 122, 832
- Vicilin, 619
  - in pea, 747
- Victoria yellow, 303
- Vignin, 179
- Villavecchia test, 576
- Villi, absorption by, 347
- Vinegar, 27, 28, 34, 898–900
  - composition, 899, 900
  - in curing, 453
  - rope, inhibition of, 716, 717
  - standards, 898
  - types, 898
- Violanin, 318
- *Viola tricolor*, 318
- Virus, 172, 177, 224
  - Bushy stunt, 162
  - Protein, 214
  - Tobacco mosaic, 162, 172, 214
- Viscosimeter, 20, 21, 22, 648. See also *Plastometer.*
  - falling ball, 21
  - Ostwald pipet, 21
  - torsion, 21
- Viscosity, 11, 19–23, 33, 40, 41, 43, 44, 45, 46, 47, 399
  - factors affecting, 20–21, 400
  - of flour suspensions, 648
  - measurement of, 21–23
  - of oils, 583
- Vitagen, 2, 6, 255–260, 270
  - amino acids, 256–258
  - carbohydrates, 258–259
  - choline and related compounds, 259
  - definition, 229
  - fatty acids, 256
  - organic, sulfur-containing compounds, 260
- Vitamin(s), 2, 6, 10, 48, 93, 105, 224, 225, 226, 229–255, 270, 272, 351, 362, 487. See also under specific vitamin.
  - group A, 230–231, 569, 585. See also *Vitamin A.*
  - B complex. See *Vitamin B complex.*
  - bacterial, 370
    - and enzymes, 224–226
  - group D, 230, 245–250, 569, 585. See also *Vitamin D*, D_2, D_3.
  - group E, 230, 250–252, 569. See also *Vitamin E.*
  - enrichment with, 297–298, 662–667, 711
  - in foods. See specific food and specific vitamin.
  - and hormones, 224–226
  - group K, 230, 253–254, 569. See also *Vitamin K*, K_1, K_2.
  - nonidentified, 255
- Vitamin A, 229–230, 233, 234, 464, 623. See also *Vitamins, group A.*
  - in blueberries, 765
  - in corn, 636
  - in fish, 488–489
  - fruits, 769, 771
- *Vitamin A* (*contd.*)
  - in milk, 405, 407, 408
  - in soybean, 745
    - flour, 682
  - in vegetables, 736, 742, 748
- Vitamin B complex, 224, 225, 230, 623
  - in bread, 711
  - in cereal grains and products, 630, 642, 643, 644, 656, 657
    - retention in milled rice, 642, 643
  - enrichment, 663
  - Vitamin B_1. See *Thiamine.*
  - Vitamin B_2. See *Riboflavin.*
  - Vitamin B_3, 255
  - Vitamin B_4, 255
  - Vitamin B_5, 242, 255
  - Vitamin B_6, 225, 230, 239–240
  - Vitamin B_7, 255
  - Vitamin B_8, 255
  - Vitamin B_c, 255
  - Vitamin B_p, 255
  - Vitamin G. See *Riboflavin.*
  - Vitamin H. See *Biotin.*
- Vitamin C, 185, 225, 230, 244–245, 258, 348, 362, 464, 623
  - in berries, 763, 764
  - in fish, 491
  - in fruits, 740, 766, 769, 771, 773, 774, 775, 776, 777, 780
  - in legumes, 743, 744, 747
  - in milk, 405, 408
  - in mushrooms, 758
  - in nuts, 761
  - in vegetables, 732, 733, 734, 737, 738, 739, 740, 742, 748, 749, 751, 752, 756
- Vitamin C_2, 255
- Vitamin D, 100, 278, 298, 623. See also *Vitamins, group D.*
  - in bread, 711
  - enrichment of flour, 662–667
  - in fish, 488–489
  - in milk, 405, 408, 415
  - in oats, 638
- Vitamin D_1, 247
- Vitamin D_2, 100, 247, 248, 249
- Vitamin D_3, 247, 248, 249
- Vitamin E, 623. See also *Vitamins, group E.*
  - activity, 621
  - in milk, 405, 408
- Vitamin I, 255
- Vitamin J, 255
- Vitamin K, 230, 254. See also *Vitamins, group K.*
- Vitamin K_1, 253
- Vitamin K_2, 253
- Vitamin L_1, 255
- Vitamin L_2, 255
- Vitamin M, 255
- Vitamin P. See *Citrin.*
- Vitazymes, 226
- Vitellin, 112, 117, 619
- *Vitis*
  - *labrusca*, 772
  - *vinifera*, 318, 765, 772

Tuna
  whi
  yell
Tung
Turbo
Turke
Turm
Turni
  carl
  com
Tyran
  oxid
Tyros
Tyros

l-Tyro
Tyrosi

Ultrac
Ultrafi
Ultran
Ultrav
  prev
Uracil,
Urea,
  from
Urease
  crys
  unit,
Uric a
Uricas
Urinar
Urine,
Urobili
Urocan
Uronic
  in tr
  units
Urtica

Vaccin
  macr
  myrt
Vagoto
Valine,

Vanadi
Vanilla
  bean
  extra
    ad
    co
  ferm
  flavo
Vanilla
  fragr
  plani
  tahite
Vanillir

W

Walden inversion, 67
Walnut, black, 761
    composition, 779
  butternut, 761
  carbohydrate content, 783
  composition, 812
  English, 761
    composition, 779
  Persian, 761
Warburg, coferment of, 170
  oxidizing fermentation enzyme, 218
  reducing fermentation enzyme, 218
  respiratory ferment, 216
  zwischenferment, 217
  zwischenferment + coenzyme II, 218
Water, 26, 27, 28, 271
  absorption of, 348–349
  alkaline, 295
  in bakery products, 710
  bound, 41, 273
  in breakfast foods, 678–679
  in cereal feeds, 631, 632
  in cereal grains and products, 629, 630, 631, 632, 644, 646, 655
  in coffee, 844
  in various flours, 655, 682
  and food spoilage, 372–373
  in fruits, 770, 775, 778–779
  in meat, 445, 446
  metabolic, 273
  in milk and milk products, 404, 405, 409, 412, 423, 425, 430, 431, 433
  mineral, 28, 295
  in mushrooms, 757
  in nuts, 778–779
  salt, 295
  sea, 27, 28
  in sirups and sugars, 818
  soda, 7
  in vegetables, 741, 748, 750, 753, 757–758
Watercress, carbohydrate content, 783
Watermelon, 122, 774
  carbohydrate content, 783
  composition, 779
Waxes, 88, 90, 98–100, 487, 774
  in cabbage, 737
  in soybean, 745
  sterols from, 98–100
Westphal balance, 11
Whale oil, 91, 565, 566, 567, 570, 571, 577, 584, 589
  characteristics, 582
  composition, 581
  liver oil, 234
Wheat, 10, 41, 111, 112, 127, 179, 591, 597–600, 604–607, 649–677
  albumin, 117, 618, 620
  amino acids in, 618
  botanical classification, 592
  bran, 599, 654, 655, 669
    amino acids in, 618
    composition, 629, 632, 655
    mineral content, 279, 630
Wheat (*contd.*)
    production, 649
    vitamin content, 630
  breakfast foods from, 678–679
    composition, 629
    flakes, composition, 629
    germ, composition, 629, 655
      mineral content, 630
      vitamin content, 630
    meals, composition, 629
    puffed, composition, 629
    shredded, composition, 629, 783
  by-product feeds, definitions, 669
  carbohydrate content, 783
  club, food products, definitions an
    667, 668
    red, 597
    white, 597, 603, 606
  commercial classification, 603–607
  common, 597
    and flour quality, 650–654
    food products, definitions and sta
      668
    and mill products, 650
  composition, 615
  cracked, 662, 668, 677
  crushed, 668
  durum, 598, 603, 605, 624, 669–673
    amber, 597, 605, 606, 670
      hard, 603, 605
    composition, 670
    definitions, 673
    flour, 673, 674
    kernel, 670
    red, 597, 598, 603, 605, 606, 670
    standards, 673
  farina, 662, 668 670, 677
  germ, 112, 599
    composition, 281, 284, 655
    and enrichment, 664
    meal, composition, 632
    oil, 94
      unsaponifiable fraction, 622
  gliadin, 614, 620, 650
  globulin, 618, 620
  gluten, 618, 620, 650–652
  glutenin, 614, 620, 650
  Humpback, 603
  kernel, 599, 626
    structure, 598–600
  leucosin, 614, 620
  malted, 691, 695, 696. See also *Flou*
  middlings, 669. See also *Flour.*
  mill streams and products, 654–658,
    chemical composition, 654–658
    coarsely ground, 662, 668
    mineral content, 656
    vitamin content, 656
  mineral constituents, 279, 288, 627,
  mixed, 603
    feed, 669
  nucleoproteins, 620
  patent. See *Flour.*
  production, 593, 597

***at** (**contd.**)*
  rolamin, 618, 620
  protein content, factors affecting, 616–617
  d dog 632, 655, 669
  piration, 614
  reenings, 632
  orts, 632, 655, 669
  onora, 603
  pring, Dark Northern, 603, 604
    Hard Red, 597, 598, 603, 617, 624, 652, 653
    Northern, 603, 604
    Red, 603, 604
  andards, U. S. grain, 603–607
  ticum species, 592, 597
  il[illegible]tion, 649, 650
  iscosity, 22
  tamins in, 624, 625, 626, 630
  hi[illegible], 597, 603, 653
    Hard, 603, 604, 606
    Soft, 603, 606
    Western, 603, 606
  inter, Dark Hard, 603, 605
    Hard, 603, 605
    Hard Red, 597, 603, 605, 624, 652, 653
    d, 603, 606
    ft Red, 597, 603, 606, 617
    estern Red, 603, 606
    llow Hard, 603, 605
  flour, 128, 659–663
  lase activity, 654
    content, 654, 655
  nded and prepared, 659–662
  nriched, 662–667
  phosphated, 659, 668
  plain, 659, 668
  eady-mixed, 661
  elf-rising, 660, 668
  mated white, 658, 668
  rbohydrate content, 783
  finitions, 668
  riched, 662–667
    mineral content, 630
    mineral requirements, 666–667
    vitamin content, 630
    vitamin requirements, 666–667
  adin, 650
  ten, 650–652, 653
  tenin, 650
  aham, 659–663
    composition, 629, 632
    production, 649
  rd, 652
    composition, 655
  gh-protein, 653, 658
  rovers, 652
  -extraction, 665, 666, 667
  ted, 659. See also *Wheat.*
  dlings, 669
  mposition, 632, 655
  nt, composition, 629, 655, 656, 658
  duction, 649
  teins and gas retention, 694
  lity, 650–654
  rising, 660, 668

***Wheat flour** (**contd.**)*
    composition, 629, 632
    production, 649
  soft, 652, 653, 701
  streams, composition, 655
  strong, 652
  weak, 652
  white, composition, 629, 632
    mineral content, 630
    production, 649
    vitamin content, 630
  whole, 659, 663, 668
Whey, 40, 432
  specific heat, 400
Whitefish, 480, 510
Whiting, 476, 483, 488, 500, 510
Wines, 28
Woad, 318
Wohl reaction, 83
Wool, 472
  fat, 95, 99
  keratin, 117
Wrapping, 720. See also ***Moistureproof wrapping.***

X

Xanthine, 172, 210, 223
  oxidase, 185, 199, 210, 217, 223
Xanthophyll, 321, 737
Xanthoproteic reaction, 152
Xylan, 68
Xylosan, 51, 68
Xylose, 58, 73, 350, 786, 796
D^+-Xylose, 51, 56, 61, 70, 78
L^--Xylose, 56, 58, 61, 70
Xyphias gladius, 489

Y

Yeast, 52, 98, 99, 100, 127, 149, 198, 199, 252, 357, 687, 689–690, 691, 692. See also ***Bakery products, Dough, Zymase***
  brewers', 171
  carboxylase, 219
  fermentation, 800
  foods, mineral, 687
  nucleic acid, 167, 168. See also ***Nucleic Acid.***
Yeasts, 110, 134, 364, 712. See also *Yeast.*
Yellow, fast wool, 305, 312
  Martius, 303, 310
  metanil, 303
  naphthol, S, 309–310
  resorcine, 303
  sunset, FCF, 303, 313
  Victoria, 303
Yellow AB, 303, 310
  reactions, 311
  spot tests, 311
Yellow OB, 303, 311
  reactions, 311
  spot tests, 311
Yellow enzyme, 170, 221, 223
Yellowtail, 479, 482, 489
Yerba maté, 873, 874
Yogurt, 432
Yolk. See *Egg.*